HEPATOPROTECTIVE ACTIVITY OF *BASELLA RUBRA LINN* AGAINST ETHANOL INDUCED HEPATOTOXICITY IN MALE WISTAR ALBINO RATS

Dissertation submitted to

THE TAMIL NADU DR.M.G.R.MEDICAL UNIVERSITY, CHENNAI

In partial fulfillment for the award of the degree of

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in

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by

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DECLARATION

Register No. 261525005, hereby declare that this dissertation entitled, **"HEPATOPROTECTIVE ACTIVITY OF *BASELLA RUBRA LINN* AGAINST ETHANOL INDUCED HEPATOTOXICITY IN MALE WISTAR ALBINO RATS"** has been originally carried out by me under the guidance and supervision of Prof. Dr.P.Amudha, M.Pharm,. PhD, Asst professor for the department of pharmacology, C.L. Baid Metha College of Pharmacy, Chennai-97 for the academic year 2016-2017. This work has not been submitted in any other degree at any other university.

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LIST OF TABLES

LIST OF ABBREVIATIONS

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase
CAT	Catalase
Conc	Concentration
DMSO	Di methyl sulfoxide
DPPH	2, 2-diphenyl-1-picrylhydrazyl
EDTA	Ethylen diamine tetra acetic acid
GGT	Gamma-Glutamyltransferase
GSH	Glutathione
MDA	Malondialdehyde
NAD*	Nicotinamide adenine dinucleotide (Oxidised)
NADH	Nicotinamide adenine dinucleotide (reduced)
EEBR	Ethanolic extract Basella rubra
ROS	Reactive oxygen species
SGOT	Serum Glutamic oxaloacetate Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SOD	Superoxide Dismutase
TLC	Thin layer chromatography
TMS	Tetramethylsilane
UV	Ultra violet
WHO	World Health Organization

µg	Microgram
µm	Micromloar
mg	Milligram
SEM	Standard error of mean
v/v	Volume by volume
w/w	Weight by weight

1. INTRODUCTION

Early in the twenty century herbal medicine was a prime healthcare system as antibiotics or analgesics were not available. With the development of allopathic systems of medicine, herbal medicine gradually lost its popularity among people and it was based on the fast therapeutic actions of synthetic drugs. Almost a century has passed and we have witnessed limitations of allopathic systems of medicine. Lately herbal medicine has gained momentum and it is evident from the fact that certain herbal remedies peaked at par with synthetic drugs.

It can be concluded that knowledge of Alternative and Complementary Systems of Medicines like Ayurveda, botany, pharmacognosy and phytochemistry, biochemistry, ethno pharmacology and toxicology is integral part of herbal medicine.

Recently we have witnessed explosive growth of herbal drug industry. Data and meta-analysis have shown that more and more people are consulting herbal practitioners. It's cheering that the World Health Organization has also identified importance of herbal medicine. According to a study from U.S., 60-70% patients living in rural areas are dependent on herbal medicine for their day to day diseases**.** (Singh A; 2007)

Several authors have reported favorable results with herbal drugs (mostly in form of extracts) either in animal or in human studies. Ginkgo biloba L., Echinacea purpurea L., Hypericum perforatum L. and Cimcifuga racemosa (L.) Nutt, were subjected to clinical trials.

Silybum marianum L., the reputed hepatoprotective, has remained a golden standard in the treated of liver ailments. Several years have passed but status of this herbal drug remains unquestioned. In India, a study reported that Picrorrhiza kurroa Royle. Is more potent than Silybum marianum as hepatoprotective agent (however, this study is not complete in all aspects). (Singh A; 2007)

Herbal drugs are significant source of hepatoprotective drugs. Mono and poly-herbal preparations have been used in various liver disorders. According to one estimate, more than 700 mono and poly-herbal preparations in the form of decoction, tincture, tablets and capsules from more than 100 plants are in clinical use. Surprisingly, several studies have appeared in journals

addressing hepatotoxic potential of herbal drugs. These studies suggest that the drugs that were claimed to be hepatoprotective are actually hepatotoxic.

In India, several steps have been taken to improve quality of Ayurvedic medicines. Good manufacturing practice (GMP) guidelines have been introduced so as to ensure quality control. Medicinal plant boards have been constituted at state and center level to inspire people, particularly the farmers for adopting cultivation of medicinal plants. Herbal gardens have been developed to make the common man conversant with the rich heritage of Indian system of medicine. Various institutes like NIPER, NBRI, CIMAP and CDRI are playing pivotal role in laying down standards for Ayurvedic system of medicine.

To conclude it may be said that herbal drugs have provided us with potent weapons like atropine, codeine, taxol, vincristine and vinblastine. In the modern scenario, diseases are becoming drug-resistant and scientists are studying possible roles of plant based drugs for screening life saving drugs. The herbal system of medicine is a fully fledged system of medicine and it cannot be ruled out as quackery. Backing up this system is the fact that ancient findings and documentation have through the centuries provided us with leads on the development of life-saving drugs.

Treatment options for common liver diseases such as cirrhosis, fatty liver, and chronic hepatitis are problematic. The effectiveness of treatments such as interferon, colchicine, penicillamine, and corticosteroids are inconsistent at best and the incidence of side-effects profound. All too often the treatment is worse than the disease. Conservative physicians often counsel watchful waiting for many of their patients, waiting in fact for the time when the disease has progressed to the point that warrants the use of heroic measures. Physicians and patients are in need of effective therapeutic agents with a low incidence of side-effects. Plants potentially constitute such a group.

Several hundred plants have been examined for use in a wide variety of liver disorders. Just a handful has been fairly well researched. The latter category of plants include: Silybum marianum (milk thistle), Picrorhiza kurroa (kutkin), Curcuma longa (turmeric), Camellia sinensis (green tea), Chelidonium majus (greater celandine), Glycyrrhiza glabra (licorice), and Allium sativa (garlic). This review will be divided into two parts. Silybum marianum and Picrorhiza

kurroa will be reviewed in Part One. Curcuma longa, Camellia sinensis, Chelidonium majus, Glycyrrhiza glabra, and Allium sativa

There are number of phytoconstituents from plants which have exhibited antihepatotoxic activity

A number of recent reviews have focused on the adverse effects of herbal products. In the current review, we will highlight on herbs known to be hepatoprotective, mechanisms of hepatoprotectivity, and clinical documentation. In fact some herbal products claiming to be Hepatoprotective may actually be having some components with hepatotoxic potential.

Silybum marianum, Picrrorhiza kurroa, Andrographis paniculata, Phyllanthus niruri, and Eclipta Alba are proven Hepatoprotective medicinal herbs, which have shown genuine utility in liver disorders. These plants are used widely in Hepatotprotective preparations and extensive studies have been done on them. Their discussion is beyond the scope of the article.

India is known as a botanical garden in world and the largest producer of herbal medicines. India recognizes more than 3000 plant as medicinal use. It is estimated that more than 6000 plants in India are in use in traditional and herbal system of medicines. Herbal medicines are used in various forms in indigenous system such as Unani, Ayurveda, and Siddha.(Farnsworth NR et al;1991)

Around 25,000 effective herbal formulations are used in traditional and folk medicine in India. The demand for plant products is increased throughout the world and the pharmaceutical companies are currently carrying out research on plant material for the potential medicinal components. Even though they are not able to prove the therapeutic effects of many plants, research continues to screen the active ingredients which form the basis of drugs to fight disease like psychological disorder, neuro-developmental disorder, diabetes, cancer, AIDS and various more chronic disease.(Prakash KC et al;2007)

Herbal drug is the oldest form of health care known to mankind. Herbs had been used by all the cultures throughout the history. In modern civilization herbal drug is an integral part of the development. Primitive man observed and appreciated the great diversity of plants available to him. The most use of medicinal plant has been developed through observation of wild animal by trials and errors. As time moved on, each tribe added the medicinal power of herbs in their

area based on their knowledge. They collected the information on herbs based on the method and well-defined it in herbal pharmacopoeia. Indeed, well into the 20th century most of the pharmacopoeia of scientific medicine was derived from the herbal lore of native place. Much of the drug commonly use now a day is of herbal origin. Most civilized country USA dispensed about 25% of prescription which contains at least one active ingredient derived from plant materials. Some are made from plant extract others are synthesized to mimic the natural plant compounds.

From last five thousand years human being has relied on natural product as the primary source of medicines. However, the last two centuries have brought an explosion to understand how the natural products are produced and how they react with other organisms. The World Health Organization (WHO) estimates that 80% of the world health populations presently use herbal medicines for some aspect of primary health care (Kokate CK et al; 2011)

In recent years synthetic drugs are showing more adverse affect, to overcome this problem researchers are trying to avoid this risk of those drugs. Whenever a drug is prescribed to a patient they are facing risk of side effect, so long term use of these drugs patient should be careful. But in herbal medicine the toxic effects are negligible, so the uses of herbal industry are growing up. Indian, Chinese are using plant as medicine, as whole plant or its extract. Toxicity of herbal drugs is less when compared with the synthetic medicines. (Farnsworth NR et al; 1991)

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2. REVIEW OF LITERATURE

2.1 Liver

The **liver** is a vital organ only found in vertebrates. The liver has a wide range of functions, including detoxification of various metabolites, protein synthesis, and the production of biochemical's necessary for digestion. It also plays a role in metabolism, regulation of glycogen storage, decomposition of red blood cells and hormone production.

Terminology related to the liver often starts in *hepat*- from the Greek word for liver

2.2 Structure

A human liver normally weighs 1.44–1.66 kg (3.2–3.7 lb) and has a width of about 15 cm. It is the heaviest internal organ and the 2nd largest gland in human body. Then it is located in the right upper quadrant of the abdominal cavity, it rests just below the diaphragm, to the right of the stomach and overlies the gallbladder.

Reddish - brown wedge-shaped organ with four lobes of unequal size and shape. Functional units of the liver are lobules and it is made up of hepatic cells (hepatocytes) which are the basic metabolic cells.

The liver is connected to two large blood vessels: the hepatic artery and the portal vein

The lobules are held together by a fine dense irregular fibro elastic connective tissue layer which extends into the structure of the liver, by accompanying the vessels (veins and arteries), ducts and nerves through the hepatic portal, The whole surface of the liver is covered in a serous coat derived from peritoneum and this has an inner fibrous coat (Gilson's capsule) to hitch it is firmly adhered. The fibrous coat is of areolar tissue and follows the vessels and ducts to support them.

2.3. Lobes

There are, on the surface, four lobes: right, left, caudate and quadrate. The **Falciform ligament** divides the liver into two main lobes, right and left, with the right lobe being the larger and is sub- divided into the right lobe proper, the **caudate lobe** and the **quadrate lobe.**

Gross anatomy traditionally divided the liver into two portions – a right and a left lobe, as viewed from the front (diaphragmatic) surface; but the underside (the visceral surface) shows it to be divided into four lobes and includes the caudate and quadrate lobes. (Erwin k et al; 2009)

The falciform ligament, visible on the front of the liver, divides the liver into a left and a much larger right lobe. From the visceral surface, the two additional lobes are located between the right and left lobes, one in front of the other. A line can be imagined running from the left of the vena cava and all the way forward to divide the liver and gallbladder into two halves. This line is called "Cantlie's line".

Other anatomical landmarks exist, such as the ligamentum venosum and the round ligament of the liver (ligamentum teres), which further divide the left side of the liver in two sections. An important anatomical landmark, the porta hepatis, also known as the *transverse fissure of the liver*, divides this left portion into four segments, which can be numbered starting at the caudate lobe as I in an anticlockwise manner. From this visceral view, seven segments can be seen, because the eighth segment is only visible in the parietal view. (Erwin k et al; 2009)

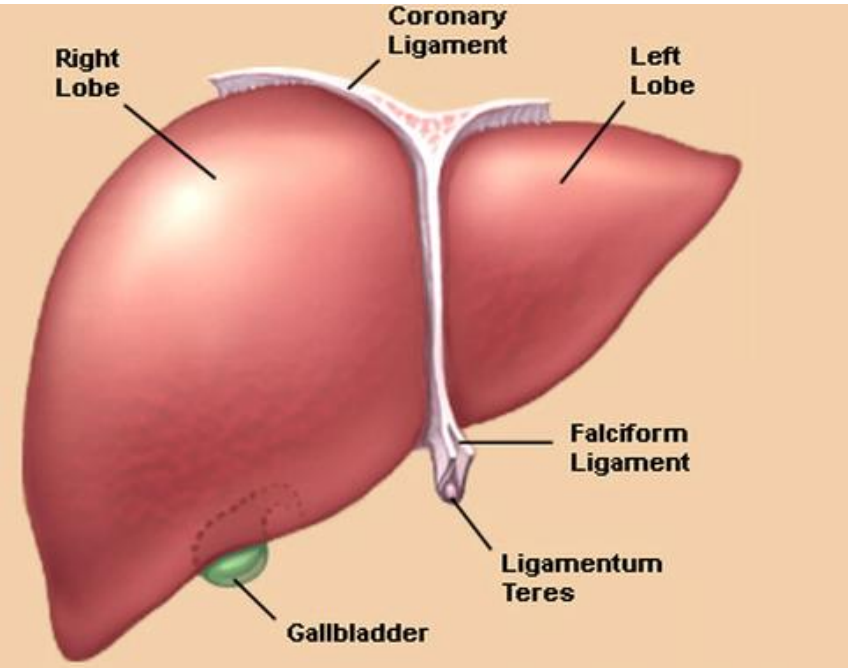


Fig. No 1: Liver lobes

2.4. Facts about liver

The liver performs over 500 different functions including fights off infection, neutralizing toxins, manufacturing proteins and hormones, controlling blood sugar and helping to clot the blood.

The liver is the largest internal and most metabolically complex organ in humans.

The liver is the only organ that can regenerate itself thus making it possible for one person to donate part of their liver to another person. When a portion of the liver is transplanted, the donor's liver will regenerate back to its original size while the transplanted portion will grow to the appropriate size for the recipient.

The liver has an enormous task of maintaining the body‘s metabolic homeostasis. This includes, the processing of dietary amino acids, carbohydrates, lipids, and vitamins; synthesis of serum proteins; and detoxification and excretion into bile of endogenous waste products and

pollutant xenobiotics. Hepatic disorders have far reaching consequences, given the critical dependence of other organs on the metabolic functions of the liver. Liver injury and its manifestations tend to follow characteristic patterns. In some instances, the diseased process is primary to the liver. In others, the hepatic involvement is secondary, often to some of the most

Common diseases in humans, such as cardiac decompensation, alcoholism and extra hepatic infections with progression of diffused disease or strategic disruption of circulation or bile flow.

1) Inflammation: Injury to hepatocytes associated with an influx of acute or chronic inflammatory cells into the liver is termed hepatitis. Attack of viable antigen- expressing liver cells by sensitized T-cells is a common cause of liver damage. Inflammation may be limited to portal tract or may spill over into the parenchyma.

E.g., viral hepatitis due to hepatitis A virus (HAV), HBV, HCV, HDV and HEV.

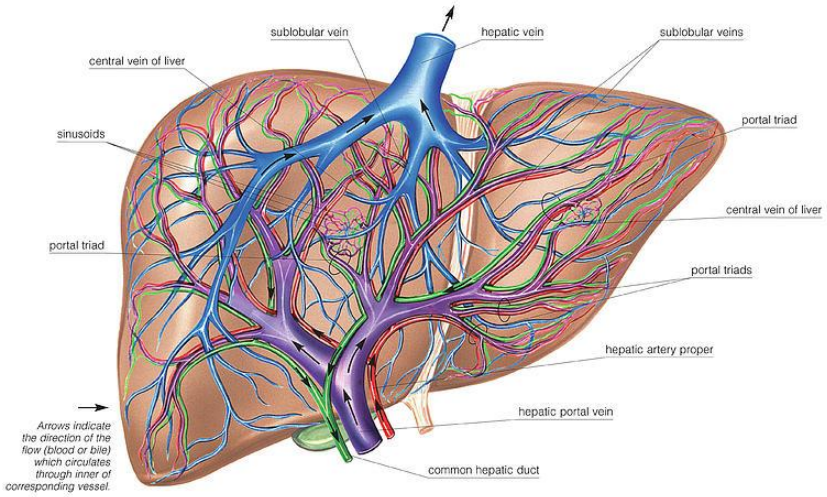


Fig 2: Liver anatomy

2) Degeneration: The hepatocytes get damaged due to toxic or immunological insult and show an edematous appearance. Degeneration can also be in the form of stetosis, where in there is accumulation of fat droplets within the hepatocytes. e.g., hepatic degeneration can be due to genetic diseases or exogenous substance such as alcohol.

3) Cell death:

Cell death which is toxic or immunologically mediated occurs via apoptosis wherein the hepatocytes become shrunken, pyknotic, and intensely eosinophilic. Alternatively, hepatocytes may also undergo lytic necrosis (osmotically swell and rupture). The other types are centrilobular necrosis, bridging necrosis, submassive necrosis and massive necrosis.

4) Fibrosis:

Fibrotic tissue is formed in response to inflammation or direct toxic insult to the liver. Deposition of collagen has lasting consequences on hepatic pattern of blood flow and perfusion of heapatocytes. Initially fibrosis may develop within or around portal tracts or the central vein or may be deposited directly with in the sinusoids. Progressively, these fibrous strands link regions of the liver (portal-to-portal, portal-to-central, central-to-central),a process called bridging fibrosis. Fibrosis is generally considered as an irreversible consequence of hepatic damage.

5) Cirrhosis:

Cirrhosis with continuing fibrosis and parenchyma injury, the liver is subdivided into nodules of degenerating hepatocytes surrounded by scar tissue, termed cirrhosis and is an end stage form of liver.

Figure 3:Cirrhosis

The clinical consequences of liver diseases are hepatic dysfunction in the form of jaundice, hypoalbuminemia, hyperammonemia, hyperglycemia, fector hepatitis, palmar erythema, spider angiomas, hypogonadism, gynecomastia, weight loss, muscle wasting, and portal hypertension from cirrhosis. If these are not treated promptly, they will lead to life threatening complications like hepatic failure in the form of hepatic encephalopathy, hepatorenal-syndrome; or portal hypertension from cirrhosis, Malignancy with chronic disease and hepatocellular carcinoma

2.5. Causes of liver disease:

Liver disease can be caused by a variety of factors. Causes include:

- Congenital birth defects, or abnormalities of the liver present at birth
- Metabolic disorders, or defects in basic body processes
- Viral or bacterial infections
- Alcohol or poisoning by toxins
- Certain medications that is toxic to the liver
- Nutritional deficiencies
- Trauma, or injury

2.6. Liver functions:

The liver is a metabolically active organ responsible for many vital life functions. The primary functions of the liver are include bile production, metabolic functions, blood detoxification or purification and storage of vitamins and minerals

The liver is thought to be responsible for up to 500 separate functions,usually in combination with other systems and organs

The liver forms part of the gastrointestinal system, which is responsible for breaking down food into smaller parts that can be used by cells. The liver is located in the abdomen,

below the ribcage. It is a large organ with many different functions, including: (Vander AJ; 2004)

- Production and secretion of bile and bile salts to help digestion and absorption.
- Production of insulin-like growth factor (IGF-I).
- Production of clotting factors.
- Release of glucose into the blood to provide energy for cells.
- Production of urea, a waste product.
- Cholesterol production.

Behind the liver there's a small organ called the gallbladder, which function's to store bile produced by the liver and empty it into the small intestine to aid digestion and absorption.(Starr C;2008)

Bile or gall is a dark green to yellowish brown fluid, produced by the liver of most vertebrates that helps the digestion of lipids in the small intestine. In humans, bile is produced by the liver (liver bile), and stored and concentrated in the gallbladder (gallbladder bile). Volume secreted per day is about 600-1000 ml and ph is around 8 and also bile helps in the emulsification of fats.

Bile salts are derived from bile acids. These are synthesized in the liver from cholesterol by hepatocytes. The two important bile acids are cholic acid and chenodeoxy cholic acid which are produced in the liver from cholesterol.

Blood purification

Blood from the stomach and intestines is filtered by the liver. To prevent contaminants from circulating in the bloodstream, the liver removes a plethora of toxic waste from our circulation, such as bacteria, antigens, imperfect or no-longer functioning blood cells e.g. damaged leukocytes and erythrocytes

Metabolic function

1) Carbohydrate metabolism

Maintenance of normal blood glucose level:

When blood glucose is low the liver breaks stored glycogen down into glucose and release into the blood stream.

When blood glucose is high the liver converts glucose to glycogen and triglycerides (for storage).

2) Protein Metabolism

The most critical aspects of protein metabolism that occur in the liver are:

- Deamination and transamination of amino acids, followed by conversion of the non-nitrogenous part of those molecules to glucose or lipids. Several of the enzymes used in these pathways (for example, alanine and aspartate aminotransferases) are commonly assayed in serum to assess liver damage.
- Removal of ammonia from the body by synthesis of urea. Ammonia is very toxic and if not rapidly and efficiently removed from the circulation, will result in central nervous system disease. A frequent cause of such hepatic encephalopathy in dogs and cats are malformations of the blood supply to the liver called portosystemic shunts.
- Synthesis of non-essential amino acids.
- Hepatocytes are responsible for synthesis of most of the plasma proteins. Albumin, the major plasma protein, is synthesized almost exclusively by the liver. Also, the liver synthesizes many of the clotting factors necessary for blood coagulation.

3) Lipid metabolism

The liver is the center of lipid metabolism. It manufactures nearly 80% of the cholesterol synthesized in the body from acetyl-CoA via a pathway that connects metabolism of carbohydrates with that of lipids. Moreover, the liver can synthesize, store, and export triglycerides. The liver is also the site of keto acid production via the pathway of fatty acid oxidation that connects lipid catabolism with activity of the tricarboxylic acid cycle.

In the process of controlling the body's level of cholesterol and triglycerides, the liver assembles, secretes, and takes up various lipoprotein particles. Some of these particles (very low-density lipoproteins [VLDL]) serve to distribute lipid to adipose tissue for storage as fat or to other tissues for immediate use. In the course of these functions, the structure of VLDL particles is modified by loss of lipid and protein components. The resulting low-density lipoprotein (LDL) particles are then returned to the liver by virtue of their affinity for a specific receptor, the LDL receptor, found on the surface of various cells of the body, including hepatocytes. Other lipoprotein particles (high-density lipoproteins [HDL]) are synthesized and secreted from the liver. They scavenge excess cholesterol and triglycerides from other tissues and from the bloodstream, returning them to the liver where they are excreted. Thus, secretion of HDL and removal of LDL are both mechanisms by which cholesterol in excess of that needed by various tissues is removed from the circulation

4) Hematological functions (Haematopoeisis and coagulation)

1. Production of fibrinogen, prothrombin, heparin, and other clotting factors VII, VIII, IC and C.
2. Destruction of erythrocytes. (at the end of their respective life span)

5) Circulatory function

1. Transfer of blood from portal to systemic circulation
2. Blood storage (regulation of blood volume)

6) Detoxification and protective functions

1. Kupffer cells remove foreign bodies from blood (phagocytosis).
2. Detoxification by conjugation, methylation, oxidation and reduction.
3. Removal of ammonia.

Removal of ammonia

When exposed to harmful substances, toxins may enter in our body like pesticides; however it results from normal digestion.

For example, when our body digests protein, ammonia is released and your liver converts it into the less toxic substance called urea that is eliminated through urine. If any wastage it can either carried by bile into your small intestines or carried by the blood to your kidneys.

2.7. A symptom of liver diseases includes:

Symptoms may begin slowly and slowly get worse. They may also begin suddenly and be severe from the start. (Nevah MI et al; 2016)

Early symptoms may be mild and include:

- Breath with a musty or sweet odor
- Change in sleep patterns
- Changes in thinking
- Confusion that is mild
- Forgetfulness
- Mental fogginess
- Personality or mood changes
- Poor concentration
- Poor judgment
- Worsening of handwriting or loss of other small hand movements

More severe symptoms may include:

- Abnormal movements or shaking of hands or arms
- Agitation, excitement, or seizures (occur rarely)
- Disorientation
- Drowsiness or confusion
- Strange behavior or severe personality changes
- Slurred speech

- Slowed or sluggish movement

People with hepatic encephalopathy can become unconscious, unresponsive, and possibly enter a coma.

A rare but severe form of the liver infection called acute fulminant hepatitis causes liver failure. Symptoms of liver failure include:

- An enlarged and tender liver
- Enlarged spleen
- Susceptibility to bleeding
- Encephalopathy, which is a disorder that affects how the brain functions
- Changes in mental status or level of consciousness
- Ascites, which is an accumulation of fluid inside the abdomen
- Edema or swelling under the skin
- Aplastic anemia, a condition in which the bone marrow cannot make blood cells

2.8. Diagnosis

Many further tests may also be used to support the diagnosis. These include blood tests, such as:

- Liver function tests, which are blood tests that check a wide variety of liver enzymes and byproducts
- A complete blood count (CBC), which looks at the type and number of blood cells in the body
- Abdominal X-rays
- Ultrasounds, to show size of abdominal organs and the presence of masses
- An upper GI study, which can detect abnormalities in the esophagus caused by liver disease
- Liver scans with radio tagged substances to show changes in the liver structure
- ERCP, or endoscopic retrograde cholangiopancreatography. A thin tube called an endoscope is used to view various structures in and around the liver.
- Abdominal CT scan or abdominal MRI, which provide more information about the liver structure and function

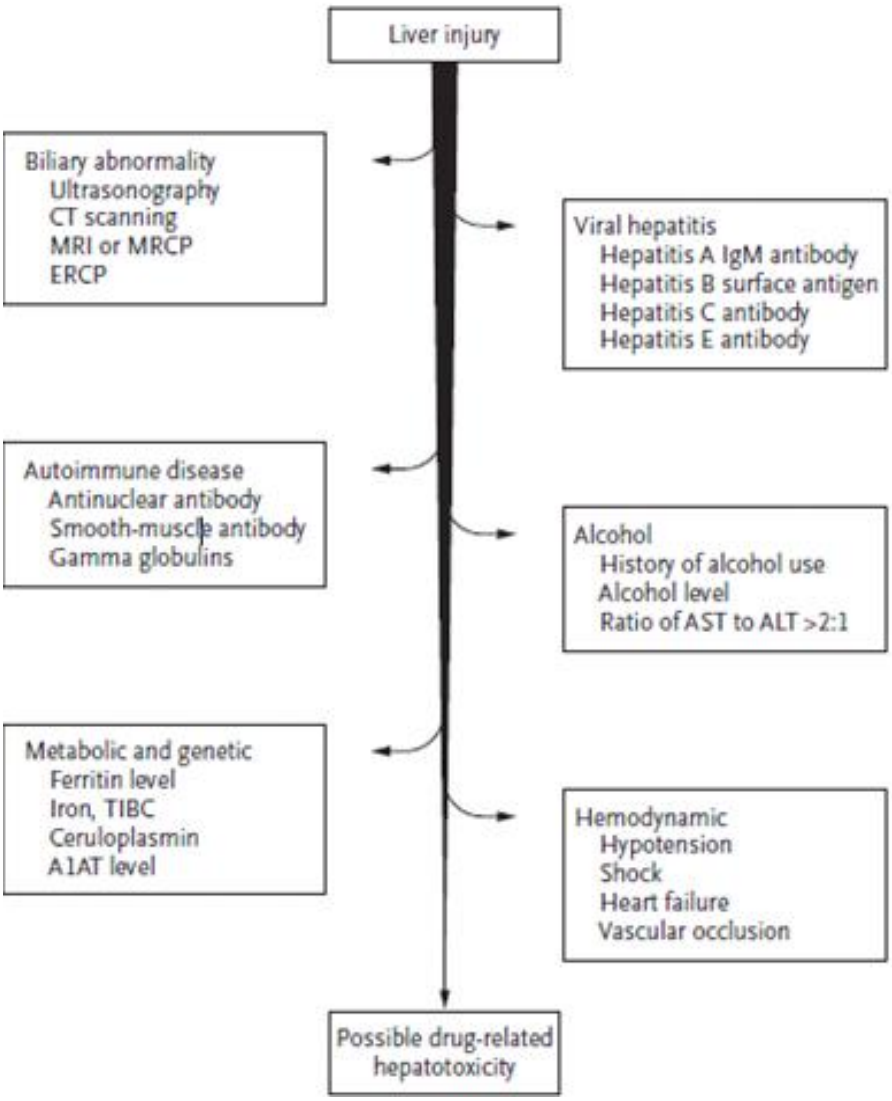


Fig No:4 Dignosis

Diagnosis of Drug-Related Hepatotoxicity.

There is no single test, including liver biopsy that can be used to diagnose drug-related Hepatotoxicity. Other causes of liver injury must first be considered with the use of a combination of serologic tests, imaging studies, and clues from the patient's history. CT denotes computed tomography, MRI magnetic resonance imaging, MRCP magnetic resonance cholangiopancreatography, ERCP endoscopic retro grade cholangiopancreatography, AST aspartate aminotransferase, ALT alanine aminotransferase, TIBC totaliron-binding capacity, and A1AT alpha1-antitrypsin

2.9. Liver disease

One way to classify liver disease is by their duration. A chronic disorder lasts for more than 6 months; a sub acute disorder lasts for 3 to 6 months, while an acute disorder occurs over a period less than 3 months. A very severe disorder that leads to liver failure within 6 weeks is termed fluminant. There are more than 100 different types of liver disease, which together affect at least 2 million people in the UK.

Diseases caused by viruses, such as hepatitis A, hepatitis B, and hepatitis C

Diseases caused by drugs, poisons, or too much alcohol. Examples include fatty liver disease, hepatic encephalopathy, and cirrhosis.

Liver cancer

Inherited diseases, such as hemochromatosis and Wilson disease.

Liver injury is defined as an alanine aminotransferase (ALT) level of more than three times the upper limit of the normal range, an alkaline phosphatase (ALP) level of more than twice the upper limit of normal, or a total bilirubin (TBL) level of more than twice the upper limit of normal if associated with any elevation of the alanine aminotransferase or alkaline phosphatase level. Liver injury is further characterized as Hepatocellular when there is a predominant initial elevation of the alanine aminotransferase level or as cholestatic when there is a predominant initial elevation of the alkaline phosphatase level; a mixed pattern comprises elevations of both the alanine aminotransferase and alkaline phosphatase levels. Recognizing the pattern of liver injury helps to categorize it, since drugs tend to create injury predominantly in one or another pattern. The injury patterns are not mutually exclusive, and a mixed pattern of injury may occur in many instances of drug-related hepatotoxicity. HAART denotes highly active antiretroviral therapy, and NSAIDs nonsteroidal antiinflammatory drugs.(Navarro VJ et al;2007)

2.9.1. Alcoholic liver disease:

It is the major cause of liver disease in Western countries. Although alcoholic steatosis (fatty liver), alcoholic hepatitis, alcoholic cirrhosis will develop in any individual who consumes a large quantity of alcoholic over a long period of time.

Fat can accumulate in the liver in excessive amounts, thus resulting in a **fatty liver;** the accumulation of fat in alcoholic steatosis may also be accompanied by a progressive inflammation of the liver (hepatitis), called **steatohepatitis**. This more severe condition may be termed either alcoholic steatohepatitis

2.9.2. Non-Alcoholic fatty liver disease:

Non-alcoholic fatty liver disease (NAFLD) is one of the types of fatty liver which occurs when fat is deposited (steatosis) in the liver due to causes other than excessive alcohol use. NAFLD has a number of causes, including being overweight, diabetes, high blood fats, and high blood pressure.

a) Non-alcoholic steatohepatitis (NASH) is the most extreme form of NAFLD.

Long term effects of the disease:

Long- term effects depend on the type of liver disease present. For example, chronic hepatitis can lead to:

- Cirrhosis of the liver
- Liver failure
- Illnesses in other parts of the body, such as kidney damage or low blood counts

Other long-term effects of liver disease may include:

- Gastrointestinal bleeding. This includes bleeding esophageal varices, which are abnormally enlarged veins in the esophagus and/or the stomach.
- Encephalopathy, which is deteriorating brain function that may progress to a coma
- Peptic ulcers, which erode the stomach lining
- Liver cancer

b) Cirrhosis

Cirrhosis is most commonly caused by alcohol, hepatitis B, hepatitis C, and non-alcoholic fatty liver disease. **Cirrhosis** is a condition in which the liver does not function properly due to long-term damage. This damage is characterized by the replacement of normal liver tissue by scar tissue. Typically, the disease develops slowly over months or years. Early on, there are often no symptoms. As the disease worsens, a person may become tired, weak, itchy, have swelling in the lower legs, develop yellow skin, bruise easily, have fluid buildup in the abdomen, or develop spider-like blood vessels on the skin. Cirrhosis is the end result of chronic liver damage.

Alcoholic cirrhosis, like all forms of cirrhosis, is often life threatening. The disease is characterized by regenerative nodules of hepatic tissue completely surrounded by fibrous scar tissue. The scar tissue grows faster than liver cells can regenerate, and the growing network of scar tissue inhibits blood flows. Once cirrhosis develops, the risk of liver cancer elevates substantially, even if the patient abstains from drinking for several years.

c) Hepatic encephalopathy

Hepatic encephalitic can occur in those with acute or chronic liver disease. Episodes can be triggered by infections, Gastro intestinal bleeding, constipation, electrolyte problems, or certain medications. This problem may occur suddenly or develop slowly over time.

Loss of brain function occurs when the liver is unable to remove toxins from the blood.it is neuropsychiatric syndrome associated with acute liver failure, chronic parenchymal liver disease or portal systemic shunting. The spectrum of symptoms may vary from subtle mental changes with recurrent disturbances in consciousness, impairment of intellectual function, neuromuscular dysfunction, elevated arterial blood ammonia concentration (Chu et al., 2001).

Hepatitis

Hepatitis is a liver disease characterize by swelling and inadequate functioning of liver. Hepatitis may be acute or chronic. In severe conditions, it may lead to liver failure and death.

Causes and Types:

Hepatitis is caused by viruses, bacteria poisons, autoimmune disease drug abuse, alcohol, some therapeutic drugs and inheritance from mother during parturition. Viral hepatitis is of five types namely, hepatitis A, B, C, D and E.

Hepatitis A and E are caused mostly by intake of water and food contaminated with hepatitis virus. Generally these two types of hepatitis are not life threatening.

Hepatitis B, C and D are caused by sharing needles with infected person, accidental prick by infected needle, having unprotected sex with infected person, inheritance from mother during parturition and blood transfusion from infected donors.

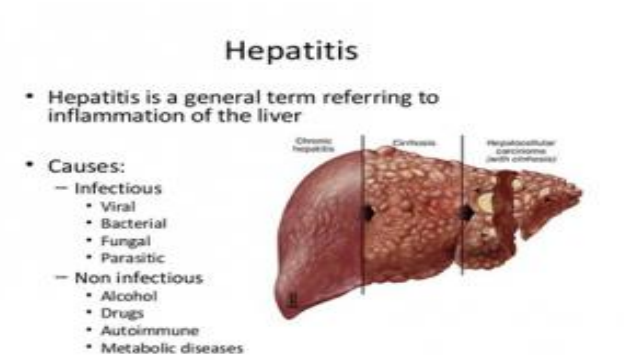


Figure 5 : Hepatitis

These three forms of hepatitis are serious diseases when compared to hepatitis A and E. Among these, hepatitis B is more common and considered more serious because it may lead to cirrhosis and cancer of liver

Alcoholic hepatitis is inflammation of the liver, and can exist as either acute or chronic conditions. Symptoms can vary greatly, from asymptomatic to severe fever, nausea, and abdominal pain. Acute hepatitis can often cause death, and the chronic form often leads to cirrhosis. On the bright side, alcoholic hepatitis is also potentially reversible, if recovery occurs and the patient abstains from drinking.

Jaundice (Sembulingam K; 2004)

This is the yellow pigmentation of the skin, mucous membrane and deeper tissues due to increased bilirubin level in blood. The normal serum bilirubin level is 0.5 to 1.5 mg%. When this serum bilirubin level exceeds 2 mg %, jaundice occurs.

Types and causes of Jaundice

Jaundice is classified into three type‘s namely haemolytic jaundice, hepatocellular jaundice, and obstructive jaundice.

a) Hemolytic Jaundice

Hemolytic jaundice is also called prehepatic jaundice. During this, the excretory function of liver is normal. But, there is excessive destruction of red blood cells and thus the bilirubin level in blood is increased the liver cells cannot excrete much bilirubin rapidly. So, it accumulates in the blood resulting in jaundice. In this type of jaundice the free bilirubin level increases in blood. Increased in formation of urobilinogen in resulting in the excretion of more amount of urobilinogenin urine. Any condition that causes hemolytic anemia can lead to hemolytic jaundice.

It represents the inability of the liver to excrete bilirubin due to various defects in the liver like

a) Criggler Najjar Disease
b) Dubin-Johnson Syndrome
c) Gilbert's disease.
d) Neonatal Physiological Jaundice.

b) Hepatocellular Jaundice

The jaundice due to the damage of liver cells is called Hepatocellular or hepatic jaundice. It is also called hepatic cholestatic jaundice. Here, bilirubin is conjugated. But the conjugated bilirubin cannot be excreted. So, it returns to the blood. The damage of liver cells occurs because of toxic substances (toxic jaundice) or by infection (infective jaundice). Commonly liver is affected by virus resulting in hepatitis.

c) Obstructive Jaundice

This is otherwise called extra hepatic cholestatic jaundice or post hepatic jaundice. It is due to the obstruction of bile flow at any level of biliary system. The bile cannot be poured into small intestine and bile salts and bile pigments enter the circulation. In this, blood contains more conjugated bilirubin.

This is caused due to obstruction of biliary tract, ductal occlusions by stones or compression by neoplastic diseases. There is an increase in the levels of conjugated bilirubin in post hepatic jaundice (Guyton and Hall, 2002)

Hemochromatosis is a condition in which too much iron is contained in the body. It is the most common genetic disease. Chronic hemochromatosis can lead to cirrhosis, cancer, impotence and heart problems. Iron damages the body through its promotion of oxidation, increasing the level of free radicals in the body. Harmful levels of iron can be accumulated in body simply by eating too of the wrong foods and supplements. The human body uses approximately I to 2 milligrams of iron daily. However, the average diet contains between 10 and 20 milligrams of iron. In hemochromatosis, the body cannot absorb iron as effectively, and also cannot detect when iron levels are too high. This excess iron is then absorbed into the body's organs, particularly the liver. Hemochromatosis is treated by lowering the level of iron in the body. The most common method is via phlebotomies. A phlebotomy is purposefully removing blood from the body. Diet is also very important to patients with hemochromatosis. Iron needs to be kept to a minimum, as well as alcohol and medications that may do further damage to the liver.

Gallstones

Cholesterol secreted by liver into the bile, may precipitate in the gall bladder to produce gallstones. Occasionally a gallstone may pass out of the gall bladder and enter the cystic duct, blocking the release of bile. Such a condition interferes with normal digestion, and often cholecystectomy is carried out (Guyton and Hall, 2002).

1.4.11. Gilbert's Syndrome

It is a fairly common, mild liver disorder. People with this disorder have a moderate, fluctuating increase in serum bilirubin, and further increase in bilirubin may produce jaundice (Crawford, 2004).

2.9.3. Inborn Errors of Metabolism

Antitrypsin Deficiency

It is an inherited condition. In this condition, the alpha antitrypsin, which is produced by the body, is abnormal with no protective activity and is not released in sufficient amount from the liver.

Wilson Disease

Wilson disease is a relatively rare hereditary condition in which excessive amounts of copper accumulate in the body including liver which leads to cirrhosis.

Hemochromatosis

Hereditary hemochromatosis refers to an HLA- linked autosomal recessive disease characterized by excessive accumulation of body iron, most of which is deposited in liver (Crawford, 2004).

Liver diseases most likely to be seen in children include:

Galactosemia: an inherited disease in which the body can not tolerate certain sugars in milk. These sugars can build up, causing serious damage to the liver and other organs of the body.

Alagille's syndrome: a condition in which the bile ducts narrow and deteriorate, especially during the first year of life

Alpha 1- antitrypsin deficiency: a genetic liver disease in children that can lead to hepatitis and cirrhosis of the liver

Neonatal hepatitis: which is hepatitis that occurs in a newborn during the first few months of life

Tyrosinemia: a disorder that causes serious problems with liver metabolism

Hemorrhagic telangiectasia: a condition in which thin blood vessels allow frequent and easy bleeding of the skin and digestive tract

Reye's syndrome: a condition that causes a buildup of fat in the liver. This condition has been linked in some cases to use of aspirin, especially in conjunction with chickenpox, influenza, or other illnesses with fever.

Wilson's disease: an inherited condition that causes a buildup of the mineral copper in the liver

Tthalassemia: a group of hereditary anemias, or low red blood cell counts

Biliary atresia: a condition in which the bile ducts extending from the liver to the intestine are too small in diameter or are missing

Chronic active hepatitis: an inflammation of the liver that causes severe scarring and interference with liver function

Like other parts of your body, cancer can affect the liver. You could also inherit a liver disease such as hemochromatosis.

2.9.4.Pediatric liver diseases

Reye Syndrome

Reye syndrome is a rare disease characterized by fatty infiltration and encephalopathy. It primarily affects children younger than 4 years. Laboratory findings include elevated blood ammonia, serum transaminases and prolonged prothrombin time.

Neonatal Hepatitis

It is an inflammation of the liver that occurs in early infancy, usually one to two months after birth. Viruses, which can cause neonatal hepatitis in infants, include cytomegalovirus, rubella (measles), and hepatitis A, B and C (Crawford, 2004).

Hepatotoxicity

A large variety of chemical compounds have identified as hepatotoxins. Liver injury induced by chemicals has been recognized as a toxicological problem for more than 100 years. "Liver injury is not a single entity; the lesion observed depends only on the chemical agents involved, but also on the period of exposure. The vulnerability of the liver to chemically induced damage is a function of

a) Its anatomical proximity to the blood supply from digestive tract

b) Its ability to concentrate and biotransform foreign chemicals.

c) Its role in the excretion of xenobiotics or their metabolites in bile (Plaa and Charbonneau, 1994).

2.9.5. Liver necrosis:

Liver necrosis is divided into 3 types.

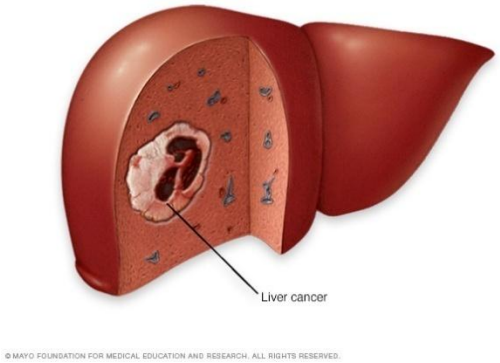


Fig No 5: Liver cancer

Diffuse necrosis:

When there is extensive and diffuse necrosis of the liver involving all the cells in group of lobules it is termed as diffuse or submassive to massive necrosis[11]. Most commonly caused by the viral hepatitis and drug toxicity.

Zonal necrosis:

It is a necrosis of hepatocytes in 3 different zones of the hepatic lobules. Accordingly it is of 3 types:

i. Centrilobular necrosis is the commonest type involving hepatocytes in zone-3. Centrilobular necrosis is the characteristic feature of ischemic injury such as in shock and CHF.
ii. Midzonal necrosis is uncommon and involves zone-2 of hepatic lobule. This pattern of necrosis is seen in yellow fever and viral hepatitis.
iii. Periportal necrosis is seen in zone-1 involving the parenchyma closest to the arterial and portal blood supply. It is almost vulnerable to the effects of circulating hepatotoxins.

Focal necrosis:

This form of necrosis involves small groups of hepatocytes irregularly distributed in the hepatic lobule. Focal necrosis is generally caused by microbiological infections.

2.10. Risk Factor of hepatotoxicity

Several conditions are Responsible for development of Hepatotoxicity. Liver injury involve certain risk factors that can be genetic, non genetic or environmental [41]. Adverse effect of drug or metabolites (like, antiretroviral drug nad alcholol) and certain health condition such as age, sex, disease (HIV or diabetes) are coupled with each other [42]. Some Resent research on hepatic injury and various disease condition Show increased hepatotoxicity among HIV, Diabetic or Tuberculosis patient

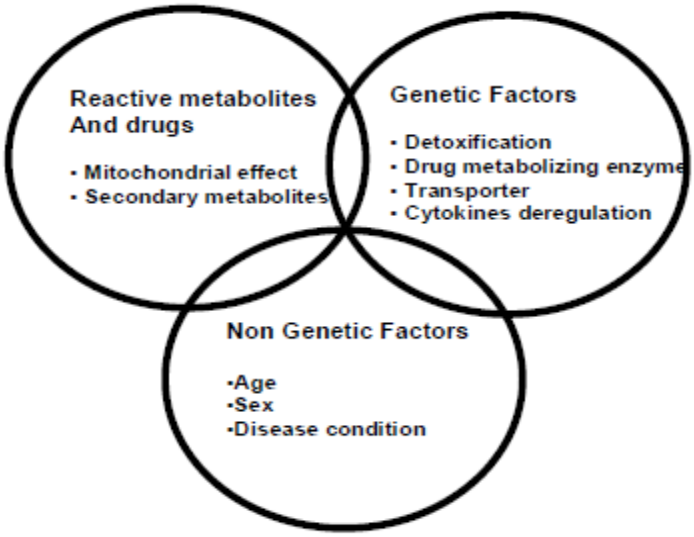


Fig No6:Risk factors

Race: Some drugs appear to have different toxicities based on race. For example, blacks and Hispanics may be more susceptible to isoniazid (INH) toxicity. The *rate of metabolism is under the control of P-450 enzymes and can vary from individual to individual.*

Age: Apart from accidental exposure, hepatic drug reactions are rare in children. Elderly persons are at *volume. In addition, poor diet, infections, and multiple hospitalizations* are important reasons for drug-induced hepatotoxicity.

Sex: Although the reasons are unknown, hepatic drug reactions are *more common in females.*

Alcohol Ingestion: Alcoholic persons are susceptible to drug toxicity because alcohol induces liver injury and cirrhotic changes that alter drug metabolism. Alcohol causes *depletion of glutathione (hepatoprotective) stores that make the person more susceptible to toxicity by drugs.*

Liver disease: In general, patients with chronic liver disease are not uniformly at increased risk of hepatic injury. Although the total cytochrome P-450 is reduced, some may be affected more than others. *The modification of doses in persons with liver disease should be based on the knowledge of the specific enzyme involved in the metabolism.* Patients with HIV infection who are co-infected with hepatitis B or C virus are at increased risk for hepatotoxic effects when treated with antiretroviral therapy. Similarly, patients with cirrhosis are at increased risk of decompensation by toxic drugs.

Other comorbidities: Persons with AIDS, persons who are malnourished, and persons who are **fasting** may be susceptible to drug reactions because of low glutathione stores.

Drug formulation: Long-acting drugs may cause more injury than shorter-acting drugs.

Host factors that may enhance susceptibility to drugs, possibly inducing liver disease

a) Female - Halothane, nitrofurantoin, sulindac

b) Male - Amoxicillin-clavulanic acid (Augmentin)

c) Old age - Acetaminophen, halothane, INH, amoxicillin-clavulanic acid

d) Young age - Salicylates, valproic acid

e) Fasting or malnutrition - Acetaminophen

f) Large body mass index/obesity - Halothane

g) Diabetes mellitus - Methotrexate, niacin

h) Renal failure - Tetracycline, allopurinol

i) AIDS - Dapsone, trimethoprim-sulfamethoxazole

j) Hepatitis C - Ibuprofen, ritonavir, flutamide

k) Preexisting liver disease - Niacin, tetracycline, methotrexate

Genetic factor

Idiosyncratic drug-induced liver injury (DILI) significantly associate with certain genetic traits and Genetic studies conducted till now abundantly have been hypothesis-driven only, Which involved a candidate compound–candidate gene approach . According to Jae Woo-kwon et al genetic variations in thioredoxin reductase 1 gene (TXNRD1) leads toward development of DILI.(Aggarwal BB et al;2007) Variation in activity of drug metabolizing enzyme, (such asCYPS3A, CYP2C9, CYP2C19) which require for drug metabolism lead to pathogenesis of idiosyncratic DILI [229]. Polymorphism of bioactivities pathway through CYP450 enzymes, detoxification reactions and excretion/transport reaction together with immunological factors (HLA class II antigen, cytokines) are major genetic factor that can effect Hepatotoxicity(Wilke RA et al;2007)

Non Genetic Factor

Environmental factors like Age, gender, Drug dose, alcohol abuse and some disease condition like HIV, and TB also associate with Hepatotoxicity. For specific medication age is important risk factor for Hepatotoxicity such as use of aspirin in younger age increase risk to liver injury [50, 51]. Large scale study on age factor in US patient has been done, shows between 25-34 age Hepatotoxicity is 4.4 par 1000 patients where as it increase vigorously to 20.83 per 1000 patient at the age of 50 or older (Lucena MI et al;2006)

Women and men have different susceptibility for drug induced hepatotoxicity. For example according to Hyman and Zimmermanin in 1978 women are more susceptible for drugs like isoniazid, nitrofurantoin, chlorpromazine etc where as men are mostly affected azathioperine induced hepatotoxicity [53]. Other factor like obesity (increased expression of CYP2E1 Associated condition), also responsible for liver injury [228] acute and chronic alcohol consumption associated hepatotoxicity has been reported in various experiment. Some disease condition like HIV tuberculosis, Hepatitis B and C associate disease always increase of liver injury.(Ekstedt M et al;2007)

2.11. Hepatotoxicity

Many xenobiotics like chemicals, Drugs, house hold things, herbs and environmental chemicals have been known to induce hepatotoxicity. Most important for xenobiotic-induced hepatic damage, the centrilobular (zone three) hepatocytes are the primary sites of cytochrome P450 enzyme activity, which frequently makes them most susceptible to xenobiotic-induced liver injury. Carbontetrachloride (CCl4), N-nitrosodiethylamine (NDEA), Acetylaminofluorene (2-AAF), Galactosamine, d-Galactosamine /Lipopolysachharide (GalN/LPS), Thioacetamide, antitubercular drugs, paracetamol, arsenic etc. are used to induce experimental hepatotoxicity in laboratory animals. Some list of chemicals is follow that are responsible for hepatotoxicity(Zimmerman HJ;1978) Industrial chemical: CCl4, Trtra chloroethane Di phenyleoxide Chloroform, Ethylene dichloride, Arsenic, Antimony, Copper, Hydrezines House hold thing: Antifreeze Dry cleaning fluids Glue, Stamping Ink Paint Products, Polishes, Paint remover, Wax Pesticides: Orgenochloride, insecticide Herbicide, fungicide Thallium, warfarin Copper salt DDT Pollutant chemical in food and water: Polychloridated Biphenyls Polybrominated biphenyls Chloroalkane Plant Extract: Pyrrolizidine alkaloids, Pennyroyal, Kava Kava, Broom corn, Bajiaolian, Margosa Oil, Jin Bu Huan, Chaparral Drugs: Paracetamol AAF

AAP, APAP, Acetophenazine Maleate AmrinoneLactate, Azacitidine, Asparaginase, Blenoxane Anti Tuberculosis drug: Isoniazid, Rifampicin, Rifabutin Pyrazinamide Ethionamide, Prothionamide Para-aminosalicylic acids

Hepatotoxicants:

A) Producing zonal Hepatocellular alterations:

Carbon tetrachloride

Chloroform

Phosphorus

Tannic acid

Ethionine

Ethanol

B) Producing bilary dysfunction

Phenothiazine derivatives

Antimicrobial agents

Anabolic steroids

Oral hypoglycemic

C) Producing Hepatocellular necrosis

Iproniazid

MAO inhibitcn

Halothane

2.11.1. Mechanisms of liver toxicity (Chun LJ et al;2009)

The pathophysiologic mechanisms of hepatotoxicity are still being explored and include both hepatocellular and extracellular mechanisms. The following are some of the mechanisms that have been described:

Disruption of the hepatocyte: Covalent binding of the drug to intracellular proteins can cause a decrease in ATP levels, leading to actin disruption. Disassembly of actin fibrils at the surface of the hepatocyte causes blebs and rupture of the membrane.

Disruption of the transport proteins: Drugs that affect transport proteins at the canalicular membrane can interrupt bile flow. Loss of villous processes and
interruption of transport pumps such as multidrug resistance–associated protein 3 prevent the excretion of bilirubin, causing cholestasis.

Cytolytic T-cell activation: Covalent binding of a drug to the P-450 enzyme acts as an immunogen, activating T cells and cytokines and stimulating a multifaceted immune response.

Apoptosis of hepatocytes: Activation of the apoptotic pathways by the tumor necrosis factor-alpha receptor of Fas may trigger the cascade of intercellular caspases, which results in programmed cell death.

Mitochondrial disruption: Certain drugs inhibit mitochondrial function by a dual effect on both beta-oxidation energy production by inhibiting the synthesis of nicotinamide adenine dinucleotide and flavin adenine dinucleotide, resulting in decreased ATP production.

Bile duct injury: Toxic metabolites excreted in bile may cause injury to the bile duct epithelium.

2.11.2. Classification of hepatotoxins:

Table 1. Classification of hepatotoxins

Category of agent	Mechanism	Histological Lesion	Examples
1. **Intrinsic toxicity** a) Direct	Membrane injury destruction of structural basis of cell metabolism	Necrosis and / or steatosis	CCl4, CHCl3, Phosphorus
	Interference with	Steatosis or	Ethionine

b) Indirect Cytotoxic	specific metabolic pathway leads to structural injury	necrosis	Thioacetamide Paracetamol Ethanol
c) Cholestatic	Intereference with bile excretory pathway leads to Cholestasis	Bile duct injury	Rifampicin Steroids
2. Host idiosyncrasy a) Hypersensitivity	Drug allergy	Necrosis or cholestasis	Sulfonamides Halothane
b) Metabolic abnormality	Production of hepatotoxic metabolites	Necrosis or cholestasis	Isoniazid

Direct hepatotoxins and their effects:

Table 2. Direct hepatotoxins and its effects

Name	Morphological alterations
Carbon tetrachloride	Decreases glycogen and protein levels and increases the content of lipid
Carbon tetrachloride-ethanol	Severely depletes glycogen level and increases

	the protein and lipid content
Thioacetamide	Decreases the glycogen and protein levels without effecting any significant change in lipid level
Paracetamol	Decreases the liver glycogen and protein contents severely and elevates lipid level
Galactosamine	Decreases the content of glycogen and protein with marked elevation in lipid profile
Fulvine	Produces edema and congestion, damaging effect of the parenchyma
Phalloidin	Damages plasma membrane of the hepatocytes, as well as their active filaments
Ethyl alcohol	Causes hepatocyte degeneration, collagen deposition and necrosis
Alfa toxins	Thymidine incorporation into DNA of regenerating liver, synthesis of RNA
Lanthanum chloride	Elevates the level of lipid , protein, α- and β globulinsin liver and decreases albumin in serum

Indirect hepatotoxins:

Drug	**Class of agent**
Methyl testosterone	Anabolic steroid
Methimazole	Antithyroid
Erythromycin estolate	Chemotherapeutic
Norethynoderal with mestranol	Oral contraceptive
Chlorpropamide	Oral contraceptive
Chlorpromazine	Tranquilizer
Tetracycline	Chemotherapeutic
Valporic acid	Anticonvulsant
Halothane	Anesthitic
Phenytoin	Anticonvulsant
Methyl dopa	Antihypertensive
Isoniazid	Chemotherapeutic
Chlorthiazide	Diuretic
Oxyphenisatin	Laxative
Paracetamol	Analgesic
Phenylbutazone	Antiinflammatory
Sulfonamides	Chemotherapeutic
Allopurinol	Xanthine oxidase inhibitor
Rifampicin	Antitubercular
Mimosa pudica Polygala chinensis (polygalaceae) Teucriumchamaedrys (Labiatae)	Anti inflammatory Sedative Atiobesity
Piper methysticum (Piperaceae)	Antiaxiety, Antipsychotic
Callilepsislaureola (Asteraceae)	Anthelmentic, Antitussive, Antiimpotence
Symphytumofficinale (Boraginaceae)	Antigastrointestinal ailments
Tussilagofarfara (Asteraceae)	Treatment of lung and gastric diseases
Lycopodiumserratum (Lycopodiaceae)	Analgesic
Tripterygiumwilfordii (Celasteraceae)	Male contraceptive
Polygonummultiflorum (Polygonaceae)	Treatment of prostate cancer and hair loss

Alcohol induced toxicity:

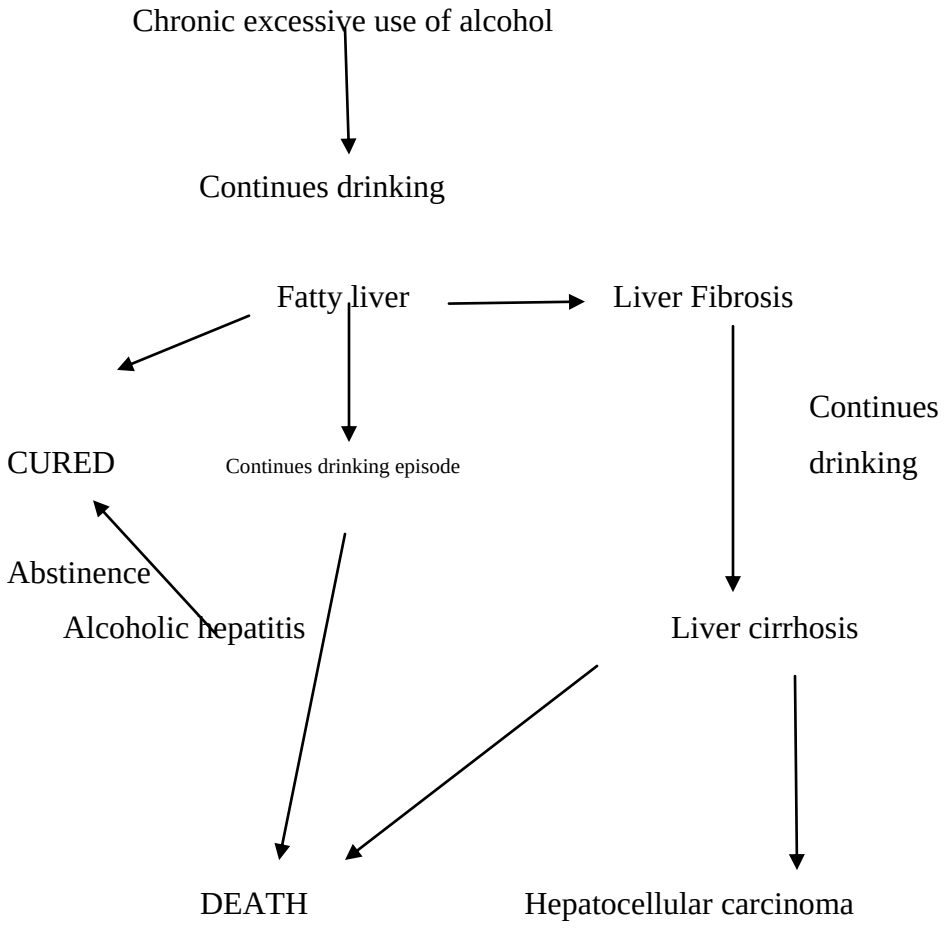


Fig : Schematic representation of Alcoholic liver diseases and progression process

Alcohol is metabolized by liver. This process produces a number of potentially dangerous byproduct.

Alcohol is converted to acetaldehyde by the enzyme Alcohol dehydrogenase (ADH).The formed acetaldehyde is highly toxic. Normally the enzyme Aldehyde dehydrogenase (ALDH) rapidly oxidizes acetaldehyde to acetate. Both these enzymes ADH and ALDH are also involved in metabolism of vitamin A.

Apart from ADH, the enzyme CYP2E1 (microsomal ethanol oxidizing system; MEOS) is also involved in metabolizing alcohol. MEOS plays a major role when blood ethanol levels are high.

CYP2E1 produces a toxic byproduct *N*-acetyl-*p*-benzo-quinone imine (NAPQI) which is responsible for damaging the hepatic protein

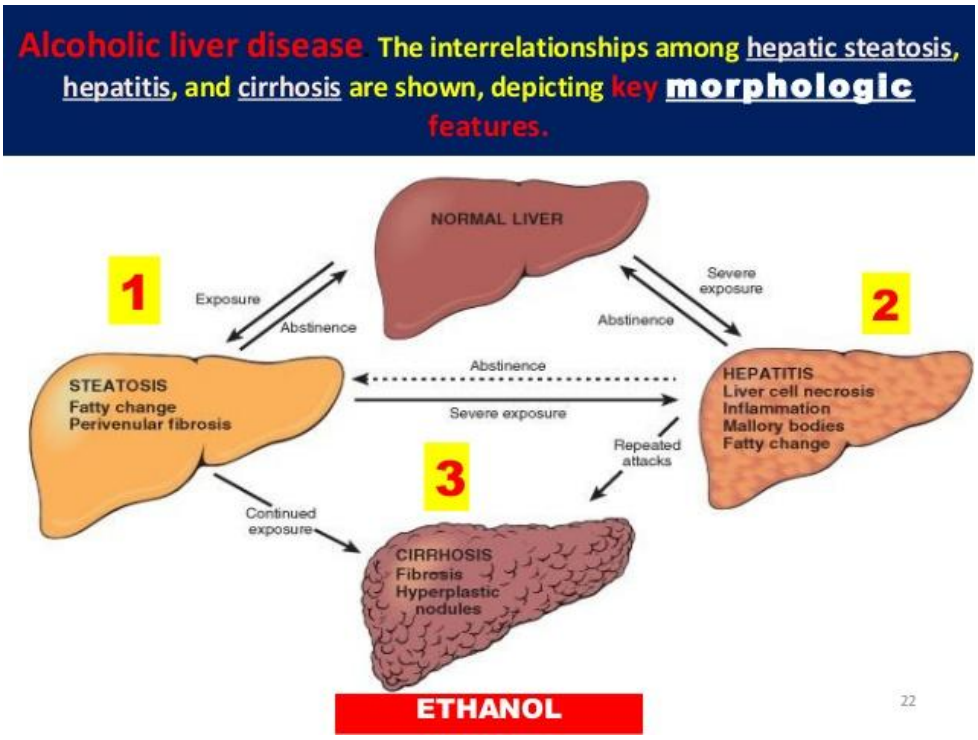


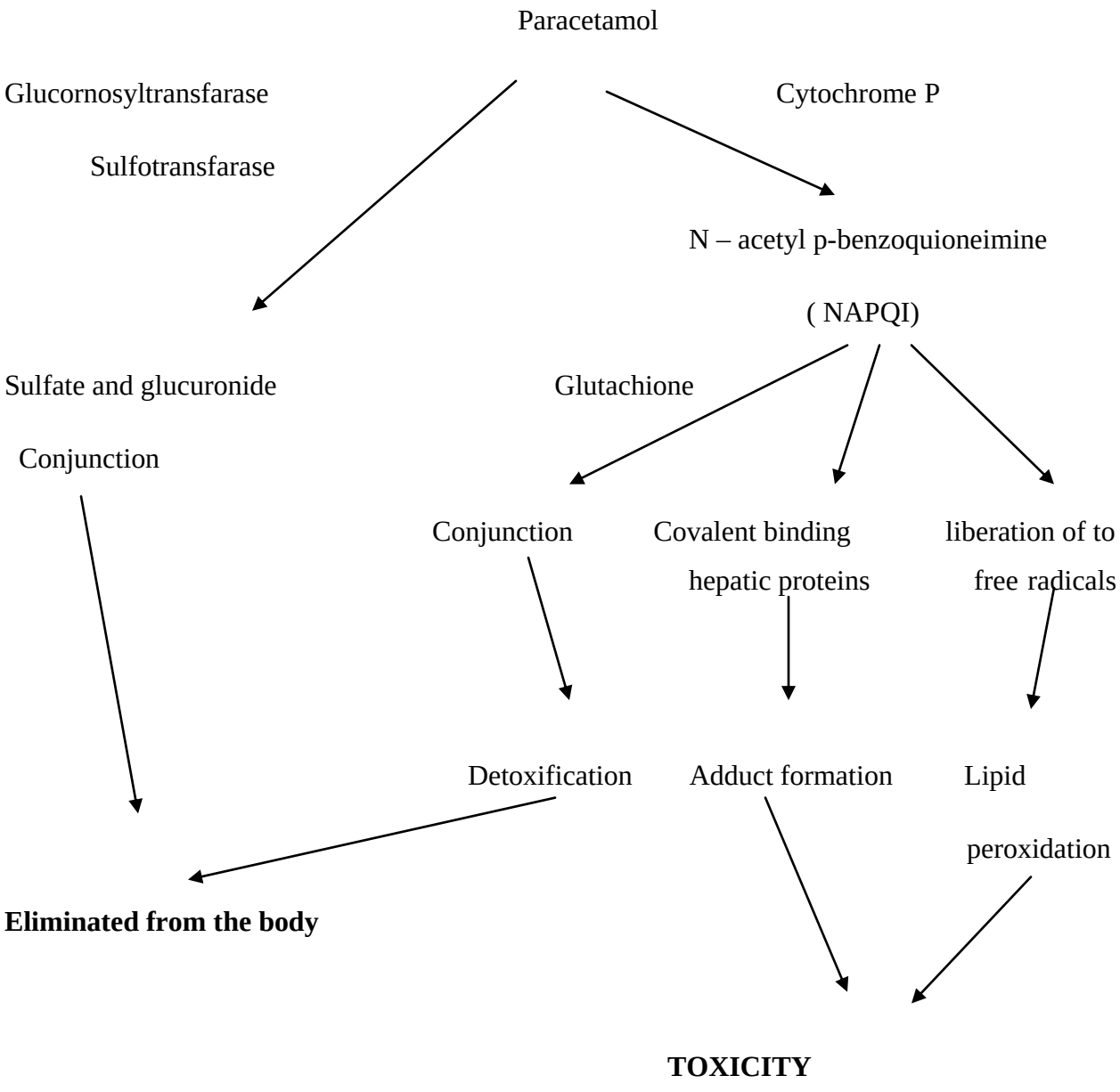
Fig No 8: Liver disease

Paracetamol induced liver toxicity:

Paracetamol is a non-steroidal anti-inflammatory drug which is available as OTC(over the counter) drug. The caution of this acetaminophen (Paracetamol) is its active metabolite is injury to liver (i.e) leads to liver damage

Normal dose of the drug- 4000mg per day (Maximum) (Franciscus A;2012)

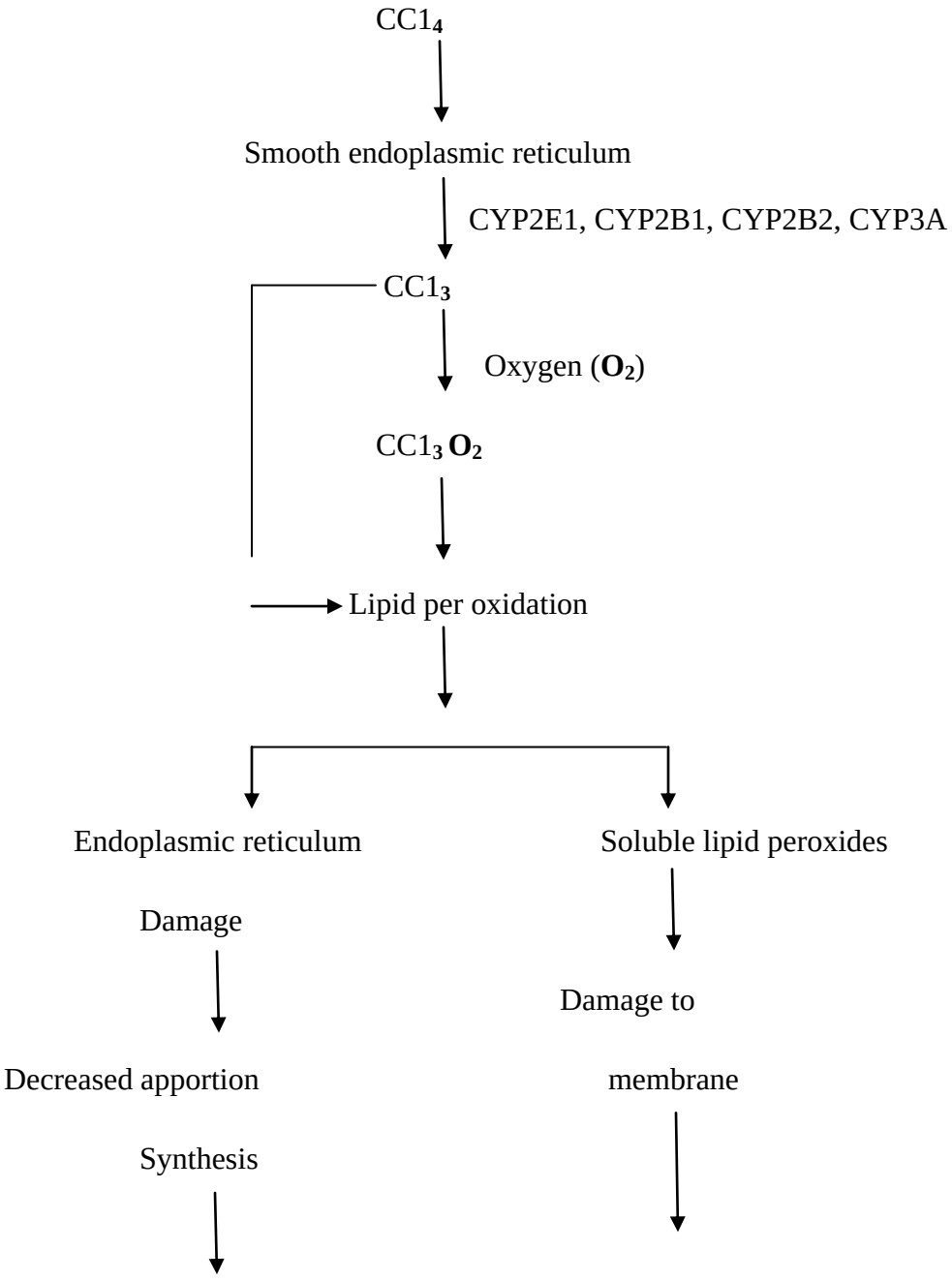
2000-3000mg per day is mostly recommended.

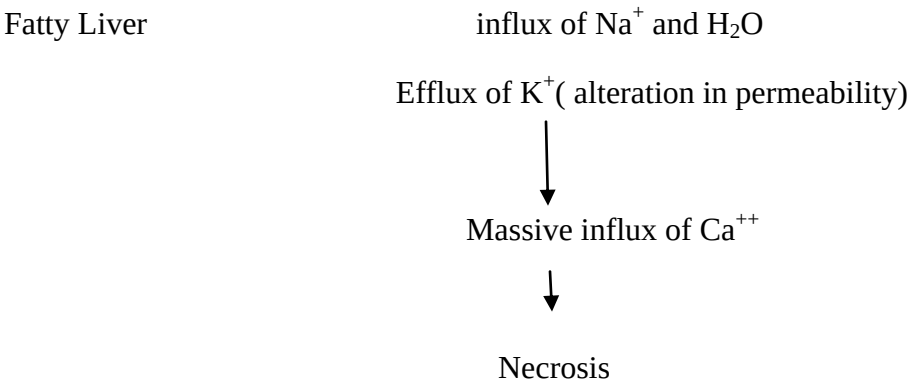


The active metabolite of acetaminophen is N-acetyl p-benzoquinoneimine(NAPQI)This NAPQI is toxic to the liver cells. Mostly the 90% of acetaminophen is metabolized by glucuronide and

sulfate conjucation and then excreted in the urine. 5-10% is metabolized by cytochrome P450, mainly by CYP2E1 which produces NAPQI.

CCl4 induced toxicity:





Carbon tetrachloride has been widely used to study liver damage used by free radicals. CCl4 toxicity is initiated by the bioactivation of CCl4 to CCl3 (tricloromethyl free radicals) by the enzymes CYP2E1, CYP2B1, CYP2B2, CYP3A chiefly by CYP2E1 (Pandit A et al)

The formed CCl3 rapidly reacts with molecular oxygen to form CCl3O2 (peroxytricloromethyl free radicals). Both CCl3 and CCl3O2 are highly reactive, they covalently bind to macromolecules such as proteins, lipids and nucleic acids and react with polyunsaturated fatty acids and form a series of self-propagating chain reactions known as “propagation of lipid peroxidation

2.12. Prevention:

Some, but not all, liver diseases can be prevented. For example, hepatitis A and hepatitis B can be prevented with vaccines.

Other ways to decrease the risk of infectious liver disease include:

• Practicing good hygiene, such as washing hands well after using the restroom or changing diapers.

• Avoiding drinking or using tap water when traveling internationally.

• Avoiding illegal drug use, especially sharing injection equipment.

• Practicing safest sex. Practicing safer sex provides less protection.

• Avoiding the sharing of personal hygiene items, such as razors or nail clippers.

• Avoiding toxic substances and excess alcohol consumption.

• Using medications only as directed.

• Using caution around industrial chemicals.

• Eating a well balanced diet following the food guide pyramid.

• Getting an injection of immune globulin after exposure to hepatitis A.

• Using recommended safety precautions in healthcare and day care work.

Animal Models in Liver Diseases

By recent advances in science and increasing knowledge of the liver pathology diseases various animal models of liver diseases has come forth,

The following table shows various hepatotoxicants induce various diseases.

TABLE 3

Agent	Type of Liver Disease
4-nitroquinolone- I-oxide	Cancer by oxidative stress (Srinivasan et al)
Alfatoxin B	Liver cancer (Mathur et.al., 1992).
Allylalcohol	Focal liver necrosis (Vogel, 2002).
Ant tubercular drugs in combination such as ionized, rifampin and pyriznamide	Drug induced hepatotoxicity to produce degeneration, necrosis and fibrosis(Kale al., 2003).
Carbontetrachloride-CC14	Centrilobular necrosis. Catty live/ Oiid (sane, 2002)
Chicken calvaria	Fibrosis (Vogel , 2002)

Concanavalin A	Autoimmune, viral and alcohol hepatitis (Zhao et al., 2004)
D-L-Galactosamine-GalN	Hepatitis (Vimal and Devaki, 2004)
Dimethyl hydrazine	Cancer by oxidative stress (Anilakumar et al., 2004)
Dimethylnitrosamine	Necrosis, fibrosis, hepatopathy and nodul regeneration and cancer in liver (Lee et al;2004)
Doxorubicin	Oxidant hepatotoxicant (Mostafa et al., 2000)
Ethyl alcohol	Fibrosis, hepatitis, cirrhosis and fatty liver (Bhandari et al;2003)
Hamster mAb (J02) against mouse Fas antigen	Fulminant liver hepatitis (Rodriguez et al;1996)
Hepatic ischemic reperfusion	Oxidative stress, post ischemic injury hepatitis (Eum and Lee, 2004)
Inhibiting protein hydroxylation*	Fibrosis (Vogel, 2002)
Lectin	Fibrosis (Davidson, 2003)
Paracetamol	Drug induced hepatitis, necrosis (Asha et 2004)
Lipopolysaccharide (LPS)	Liver sepsis (Cho et al., 2003)
siRNA (small inhibitory RNA)	Autoimmune liver disorders (Davidson, 200
Thioacetamide-TAA	Cirrhosis, fibrosis (Wardi et al., 2001)

*4 hydroxyproline confirms stability of collegenase protein liver and inhibiting p hydroxylation is possible target for the therapeutic antifibrotic agent could be evaluated (Vogel, 2002)

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2. FREE RADICALS AND THEIR SCAVENGERS

The ability to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins, and carbohydrates for energy; however, it does not come without cost. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called free radical or reactive oxygen species (ROS). About 5% or more of the inhaled 02 is converted to ROS such as O2-, H202 and OH by univalent reduction of O2 Thus cells under aerobic condition are always threatened with the insult of ROS, which however are efficiently taken care of by the highly powerful antioxidant systems of the cell without any untoward effect. When the balance between ROS production and antioxidant defence is lost, 'oxidative stress' results which through a series of events deregulates the cellular functions leading to various pathological condition

Damage to cells caused by free radicals is believed to play a central role in various human disorders like rheumatoid arthritis, hemorrhagic shock. Cardiovascular disease,

Fibrosis. Metabolic disorders, neurodegenerative disease, gastrointestinal ulcerogenosis and AIDS, Some specific examples of ROS mediated disease are Alzheimer's disease, Parkinson's disease, oxidative modification of low-density lipoprotein in atherosclerosis, cancer, Down's syndrome, and ischemic reperfusion injury in different tissues including heart, brain, kidney, liver, and gastrointestinal tract. Among these, role of ROS in atherosclerosis and ischemic injury in heart and brain studied extensively. (Bandyopadhyay U; 1999)

The involvement of free radical reactions in the pathogenesis of liver injury has investigated for many years. Increasing evidence of free radical involvement reported for chronic ethanolic intoxication and iron overload, but the most striking proof Of a role of free radical chain reactions namely lipid peroxidation in the acute lethal damage o the hepatocyte has been obtained so far in ischemic hepatitis. It is now generally accepted that free radicals can exert cellular damage through a variety of mechanisms e.g.peroxidation, covalent binding, depletion of glutathione and protein thiols, derangement intracellular free calcium homeostasis, DNA fragmentation etc.,

Estimation of phenolic and total flavanoid content

A phenol comprises the largest group of plant secondary metabolites. They range from simple structures with only one benzene ring to larger molecules such as tannins, anthraquinones, flavonoids and Coumarin. They are defined as compounds that have at least one hydroxyl group attached to a benzene ring, Phenolic compounds are plants and they have been reported to have multiple biological effects, including antioxidant activity.

The word 'Flavonoids' is derived from the Latin word flavus meaning yellow and many flavonoids are indeed yellow in color. It consists of a single benzene ring joined to a benzogamma-pyrone structure. They are able to complex metal ions, acts as antioxidants and bind to proteins such as enzymes and structural proteins. The different classes with in the groups are distinguished by additional oxygen containing heterocyclic rings and hydroxyl groups. These include the catechins, anthocyanidins, flavanols, flavones and isoflavones.

3.1. *INVITRO* STUDY

ANTIOXIDANT STUDIES:

Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction, like dominoes. Their chief danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane. Cells may function poorly or die if this occurs (Khandalwal KR)

To prevent free radical damage the body has a defense system of antioxidants. Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Although there are several enzyme systems within the body that scavenge free radicals, the principle micronutrient (vitamin) antioxidants are vitamin E, beta-carotene, and vitamin C. Additionally, selenium, a trace metal that is required for proper function of one of the body's antioxidant enzyme systems, is sometimes included in this category. The body cannot manufacture these micronutrients so they must be supplied in the diet.

Vitamin E : d-alpha tocopherol. A fat soluble vitamin present in nuts, seeds, vegetable and fish oils, whole grains (esp. wheat germ), fortified cereals, and apricots. Current recommended daily allowance (RDA) is 15 IU per day for men and 12 IU per day for women (Khandalwal KR)

Vitamin C : Ascorbic acid is a water soluble vitamin present in citrus fruits and juices, green peppers, cabbage, spinach, broccoli, kale, cantaloupe, kiwi, and strawberries. The RDA is 60 mg per day. Intake above 2000 mg may be associated with adverse side effects in some individuals

Beta-carotene is a precursor to vitamin A (retinol) and is present in liver, egg yolk, milk, butter, spinach, carrots, squash, broccoli, yams, tomato, cantaloupe, peaches, and grains. Because beta-carotene is converted to vitamin A by the body there is no set requirement. Instead the RDA is expressed as retinol equivalents (RE), to clarify the relationship. (NOTE: Vitamin A has no antioxidant properties and can be quite toxic when taken in excess) (Khandalwal KR)

DPPH radical scavenging activity:

The effect of ethanol fraction of B.Rubra on DPPH radical was estimated by (Lin et al, 2003) with minor modification. In brief, 2ml of DPPH in methanol (3.6 × 10-5 M) were added to 50 μL of various concentrations test substance (25μl-1ml). The mixture was vortexed for 15 sec and left to stand at 37℃ for 30 min. the decrease in the absorbance at 517 nm was continuously recorded in a spectrophotometer for 15 min at room temperature. All determination was performed in triplicate. The DPPH scavenging activity (decrease of absorbance at 517 nm) of extract was plotted against time and the percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 15 min duration as follows,

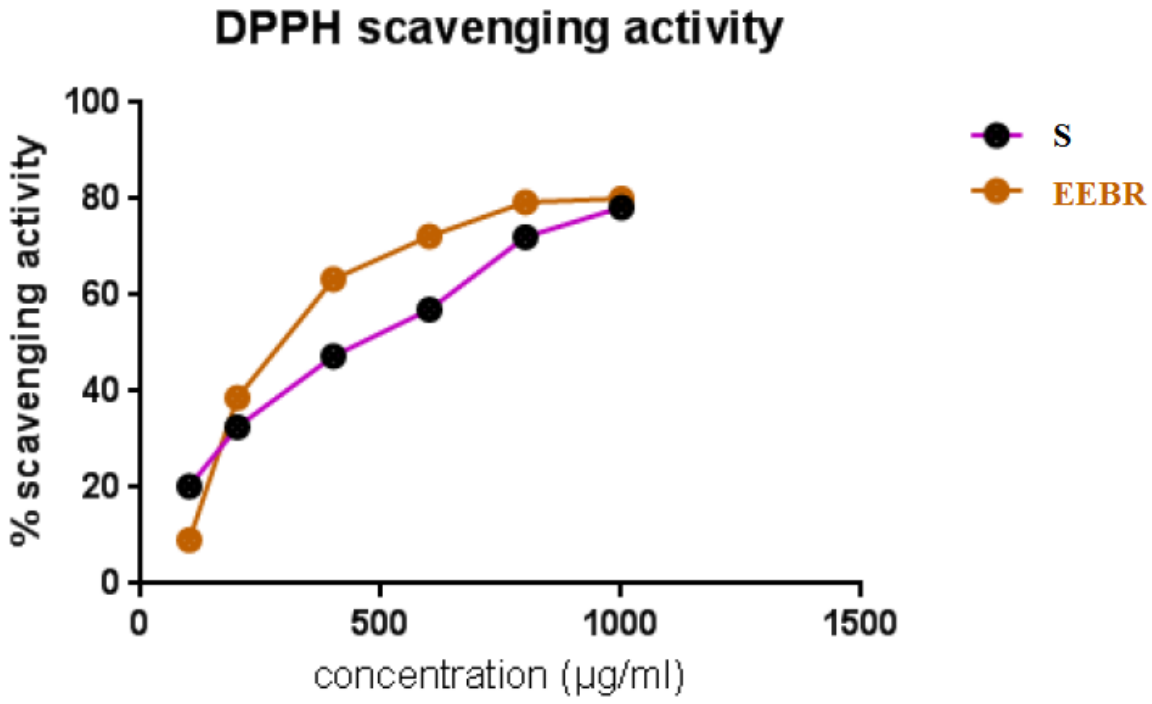
$$\% \text{ Inhibition} = (\text{Abs Control} - \text{Abs Sample}) \times 100 / \text{AbsControl}$$

Where,

Abs Control is absorbance of control at time = 0

Abs Sample is absorbance of test sample at time =15 min.

Concentration	**Silymarin**	**Ethanolic extraction**
100µg/ml	20.297±0.01	9.14±0.00
200 µg/ml	32.657±0.31	38.72±0.21
400 µg/ml	47.403±0.25	63.41±0.25
600 µg/ml	57.052±0.66	72.26±0.02
800 µg/ml	72.0840±0.79	79.34±0.45
1000 µg/ml	78.261±0.52	80.14±0.16
IC50 Value	585(µg/ml)	305 (µg/ml)



Graph of DPPH scavenging activity

S indicates Silymarin

EEBR-Ethanolic extract of Basella Rubra

Report:

In the DPPH radical scavenging assay DPPH radical was used as a substrate to evaluate free radical scavenging activity of Ethanolic extraction of *Basella rubra*. This involves the reaction between the specific antioxidant and the 2,2diphenyl picryyl hydrazide (DPPH). Silymarin was used as a standard. As a result there is a reduction of DPPH concentration by the antioxidant principle present in the extract which was confirmed by optical absorbance of DPPH; this was detected by spectrophotometer at 517nm.

4. HERBAL DRUGS

Herbal wealth of India

Now-a-days natural products are an integral part of human health care system, because there is popular concern over toxicity and resistance of modern drugs. India is one of the 12 leading biodiversity centers with presence of over 45,000 different plant species, 15000-18000 flowering plants, 23,000 fungi, 16,000 lichens, 18,000 bryophytes and 13 million marine organisms. From this flora, 15,000 to 20,000 have good medicinal value. Among those only about 7,000 plants are used in Ayurveda, 600 in Siddha, 700 in Unani and 30 in modern medicines.

Researches in isolated plant constituents are of greater importance, it has given rise to many of the world's most useful drugs. Tubocurarine, the most powerful muscle relaxant, in existence is derived from curare and the strongest pain killer of all, morphine comes from poppy cocaine from coca.

Diseases always co existed with livings, detecting their remedies also always continuing, going through the commencement of drug therapy for disease, drug comes to the force in sudden, in the ancient time human knowledge found the absence of some forms the base for the development of some disease, they were tried to use the particular disease and they got success in that work. These motivate the plant researches to use different plants, plant parts for different disease.

Our traditional system of medicines siddha categorized nearly 5000 plants species and their usage. Later on the allopathic system of medicine comes to force and dominate the siddha and due to the fast relieving nature it reaches the world as quickly and diminished the usage of plant medicine as maximum.

But allopathy system cannot provide ultimate solution to some diseases, and also their side effect in particularly the long term therapy, limits their usage still plant medicine is recommended and usage still the plant medicine is recommended and used in such cases. This suggests the plant medicine to researches as and scientific world as alternate to allopathy system of medicine. The world health organization also recognize and motivate the plant researches and centre, hence the plant medicine now considered to be alternative system of medicine.

Even usage of plants are known since plants species consist of mixture of compound, isolating the single compound and identifying the component is responsible for that particular activity is a major question in front of plant researches and also it is very difficult to say only these are all the compounds available from particular plant.

Now days due to the development of science and technology such as chromatography technique and spectroscopical technique it is possible to isolate almost all the components of plant and characterize them. Isolating and characterization are very important to improve effectiveness, minimizing the dose and on set of action.

3.2.1. A Review of Plants Used in the Treatment of Liver Disease

In recent years many researchers have examined the effects of plants used traditionally by indigenous healers and herbalists to support liver function and treat diseases of the liver. In most cases, research has confirmed traditional experience and wisdom by discovering the mechanisms and modes of action of these plants as well as reaffirming the therapeutic effectiveness of certain plants or plant extracts in clinical studies.

Several hundred plants have been examined for use in a wide variety of liver disorders.

Just a handful have been fairly well researched. The latter category of plants include: *Silybum marianum* (milk thistle), *Picrorhiza kurroa* (kutkin), *Curcuma longa* (turmeric), *Camellia sinensis*(green tea), *Chelidonium majus* (greater celandine), *Glycyrrhiza glabra* (licorice), and *Allium sativum(garlic)*

Hepatoprotective natural plants used to treat liver disease

Some of the crude drugs with activity against liver diseases are:

• Eclipta Alba (Asteraceae),
• Glycyrrhiza glabra (Leguminosae),
• Boerhaavia diffusa (Nyctaginaceae),
• Phyllanthus amarus (Euphorbiaceae),
• Silybum marianum (Compositeae),
• Uncaria gamber (Rubiaceae),
• Andrograhis paniculata (Acanthaceae).

Some of the reported constituents with pharmacologically/ therapeutically proved claims may be enlisted as, was also reported for its hepatoprotective properties.

• Silymarin
• Glycyrrhizin
• (+) –Catechin
• Saikosaponins
• Curcumin
• Picrolive I and II
• Gomosin(Wagner et al.,1998)
• Acetylbergenin (Lim et al.,2000)
• Kolaviron (Oluwatosin and Edward, 2006)

<u>Silybum marianum:</u>

• Silybum marianum is currently the most well researched plant in the treatment of liver disease.

• Also use in the dyspepsia, disorders of biliary system, liver disorder.

• It is used as Hepatoprotective and in chronic inflammatory hepatic disorders including hepatitis, cirrhosis and fatty infiltration which occur due to industrial pollutants and alcohol.

• It has also been found to be effective against liver poisioning due to alpha-galactosamine, carbon tetrachloride and tioacetamide.

• It has reported that therapeutic utility of silymarin is due to stabilization of cell membrane, stimulation of protein synthesis and accelerating the process of regeneration of hepatic cells.

• The mechanism of Hepatoprotective effect of silymarin has been suggested variously like antioxidant activity by trapping superoxide anions, stimulation of RNA synthesis and in case of amanita phalloides poisioning, blocking the receptor sites of outer liver cell membranes

• Silymarin is preferably given by parental route, due to low water solubility of flavonoligans if taken orally, only 20-50% is absorbed.

2). Taraxacum officinale:

• Hepatic & biliary disorders, kidney stones.

• Traditionally taraxacum officinale has been used as a remedy for jaundice and other disorders of the liver and gallbladder, and as a remedy for counteracting water retention.

• Generally, the roots of the plants have the most activity regarding the liver and gallbladder.

• Oral administration of extracts from the roots of taraxacum officinale has been shown to act as a cholagogue, increasing the flow of bile.

• Action: diuretic, tonic.

3). Cichorium intybus:

It is commonly known as kasni and is part of polyherbal formulations used in the treatment of liver diseases.

• In mice, liver protection was observed at various doses of Cichorium intybus but optimum protection was seen with a dose of 75 mg/kg given 30 minutes after CCl4 intoxication.

• In preclinical studies an alcoholic extract of the Cichorium intybus was found to be effective against chlorpromazine-induced hepatic damage in adult albino rats

4). Solanum nigrum:

Aromatic water extracted from the drug is widely prescribed by herbal vendors for liver disorders.

• Although clinical documentation is scare as far as Hepatoprotective activity is concerned, but some traditional practitioners have reported favorable results with powdered extract of the plant.

• It is in treatment of cirrhosis of the liver.

• Also used as a emollient, diuretic, antiseptic, and laxative properties.

• Antimicrobial, antioxidants, cytotoxic properties.

• It is also have antiulcerogenic activity and Hepatoprotective activity.

5). Glychyrrhiza glabra:

Glycyrrhizin use for anti-viral.

• It has potential for therapeutic use in liver disease.

• Experimental hepatitis and cirrhosis studies on rats found that it can promote the regeneration of liver cells and at the same time inhibit fibrosis.

• Glycyrrhizin can alleviate histological disorder due to inflammation and restore the liver structure and function from the damage due to carbon tetrachloride.

• The effects including: lowering the SGPT, reducing the degeneration and necrosis and recovering the glycogen and RNA of liver cells.

• Effects of glycyrrhizin has been studied on free radical generation and lipid peroxidation in primary cultured rat hepatocytes.

• Favorable results have been reported in children suffering from cytomegalovirus aftrer treating with glycyrrhizin.

3.2.2. Hepatic injury

Ethanol is hepatotoxic through redox changes produced by the NADH generated in its oxidation via the alcohol dehydrogenase pathway, which in turn affects the metabolism of lipids, carbohydrates, proteins and purines. Ethanol is also oxidized in liver microsomes by an ethanol-inducible cytochrome P-450 which contributes to ethanol metabolism and tolerance and activates xenobiotics to toxic radicals thereby explaining increased vulnerability of the heavy drinker to industrial solvents, anesthetic agents, commonly prescribed drugs over the counter analgesics, chemical carcinogens and even nutritional factors such as vitamin A. Induction also results in energy wastage and increased production of acetaldehyde. Acetaldehyde, in turn causes injury through the formation of protein adducts, resulting in antibody production, enzyme inactivation, decreased DNA repair and alterations in microtubules, plasma membranes and mitochondria with a striking impairment of oxygen utilization. Acetaldehyde also causes glutathione depletion and lipid peroxidation and stimulates hepatic collagen synthesis, thereby promoting fibrosis.(Liebercs;1990)

Mechanism of action of liver injury

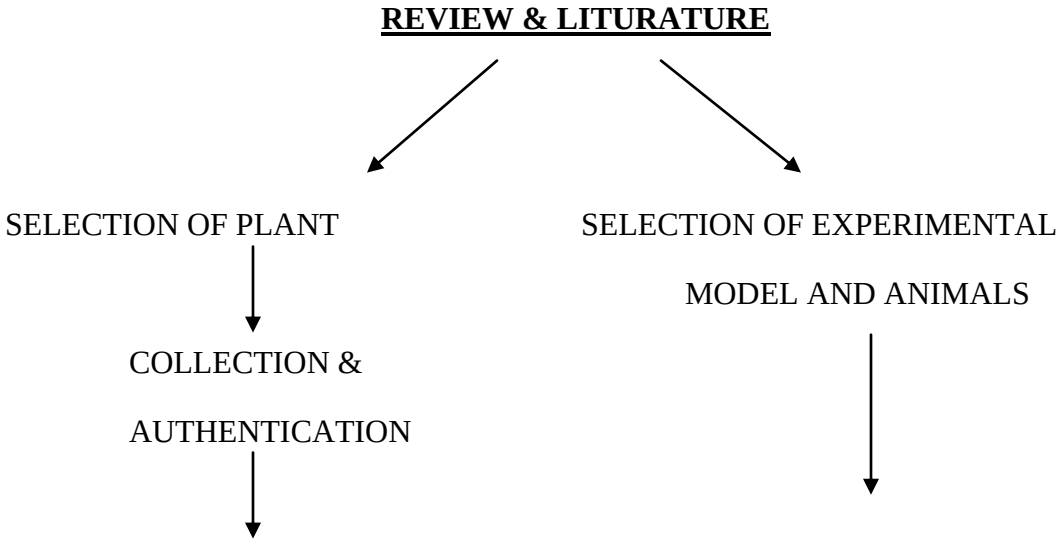
Alcohol also activates Kupffer cells sensitized by lipopolysaccharides {Enomoto et al.,1998} which promotes the production of proinflammatory cytokines, such as tumour necrosis factor [TNF]alpha, IL-1beta and IL-6 [McClain, Barve, Deaciuc, Kugelmas & Hill, Thurman et al., 1999; Tilg & Diehl,2000]. Overproduction of pro-inflammatory cytokines is known to play a critical role in the development of alcohol-induced liver injury by contributing to hepatocyte dysfunction, apoptosis and characteristic fibrosis [Bataller & Brenner, 2005; Neuman,2003]

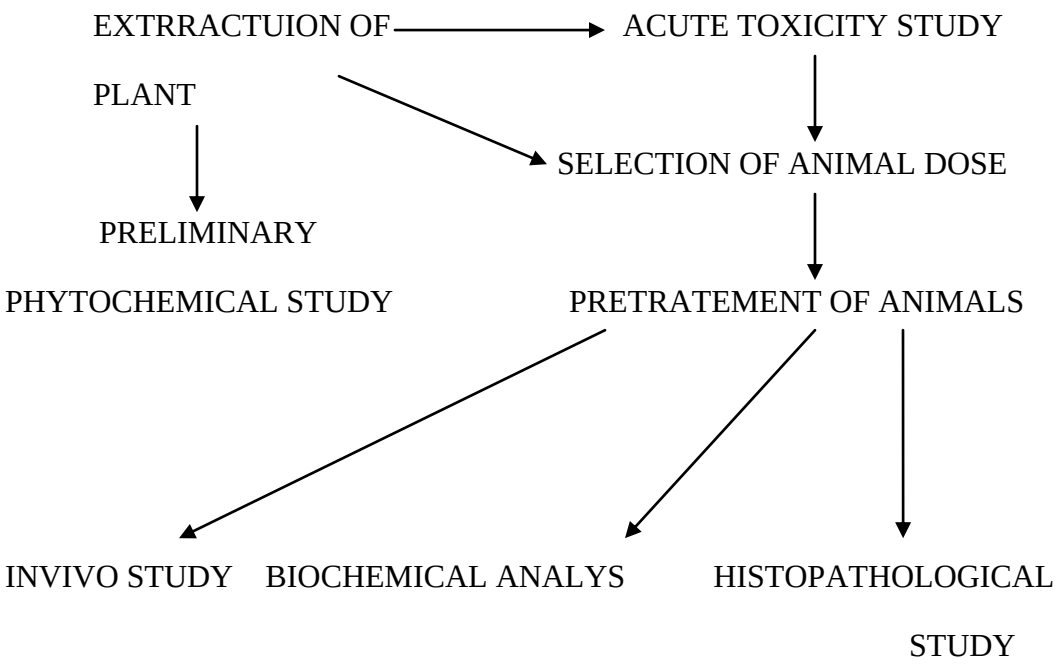
Alcohol treatment results in increases in the release of endotoxin from gut bacteria and membrane permeability of the gut to endotoxin or both. Females are more sensitive to these changes. Elevated levels of endotoxin activate kupffer cells to release substances such as eicosanoids, TNF-alpha and free radicals. Prostaglandins increase oxygen uptake and most likely are responsible for the hypermetabolic state in the liver. The increase in oxygen demand leads to

hypoxia in the liver and on reperfusion, alpha-hydroxyethyl free radicals are formed which lead to tissue damage in oxygen-poor pericentral regions of the liver lobule.

It was reported that alcohol stimulates a pro-inflammatory mediator in the liver by increasing the production of nitric oxide, an important cause of inflammation.

5. PLAN OF WORK




EXTRRACTUION OF PLANT
ACUTE TOXICITY STUDY
SELECTION OF ANIMAL DOSE
PRELIMINARY PHYTOCHEMICAL STUDY
PRETRATEMENT OF ANIMALS
INVIVO STUDY
BIOCHEMICAL ANALYS
HISTOPATHOLOGICAL STUDY

6. PLANT INTRODUCTION

Basella rubra:

Basella rubra L. (Basellaceae), commonly known as Indian or Malabar spinach belongs to family Basellaceae, is an herbaceous annual or biennial climbing herb found in tropical and sub-tropical areas. It is a succulent, branched, smooth, twining herbaceous vine, several meters in length. Stems are purplish or green. Leaves are fleshy, ovate or heart-shaped, 5 to 12 cms long, stalked, tapering to a pointed tip with a cordate base. Fruit is fleshy, stalkless, ovoid or spherical, 5-6 mm long, and purple when mature and contain only one seed and the flowers are pink.(Manju Singh et al; 2016)

Fig 9: Basella rubra

Scientific classification:

Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Eudicots
Order:	Caryophyllales
Family:	Basellaceae
Genus:	Basella
Species:	B. alba

Binomial name Basella Alba L.

Synonyms

Bengali- poi

Hindi- poi, poy, poi shak

Kannada- Basale

Marathi- Poi, Basala

Tamil- Shivappu-vasla-kire

Telugu- Batsal

Fig 10: Dried leaves of basella rubra

Fig 11: Powdered leaves

Organoleptic Character (Maheswari JK)

Sparsh (Touch)- *Snigdha (smooth)*

Rupa (Apperance) – *Dark green*

Rasa (Taste) - *Madhura*

Gandha (Smell) - No particular smell

Chemical Constituents:

The plant contains calcium 2.32, potassium 5.8, magnesium 0.06, sodium 5.11, iron 0.04mg/100gm. the leaves of the plant contain flavonoids (133.1±26.2 mg QC /100 g FM), β-cyanin and 7, 4′⁻ di–ortho methyl kempferol. The flower contain phenolic compounds (269.0±3.1mg GAE/100 g FM) such as Rutin, Quercetin, Scopoletin, Coumarin, β-xanthin and β-cyanin pigments and Caffeic, Homo-protocatechuic-, Chlorogenic-, trans- and cis-p-coumaric-, p-hydroxy-benzoic-, phloretic-, trans- and cis-sinapic-, cinnamic- acids; and the fruit consists of β-cyanin, gomphrenin I, gomphrenin II, and gomphrenin III.(Tanu shekhawat;2008)

The chemical composition of the leaf extract has been found to be: proteins, fat, vitamin A, vitamin C, vitamin E, vitamin K, vitamin B9 (folic acid), riboflavin, niacin, thiamine and minerals such as calcium, magnesium and iron. Some unique constituents of the plant are basellasaponins, kempferol and betalain.(shruthi et al;2014)

Therapeutic uses

Basella rubra has been used from a long time back for the treatment of many diseases like dysentery, diarrhea, anemia, cancer.

The paste of root of red B. rubra along with rice washed water is taken in the morning in empty stomach for one month to cure irregular periods. Leaves are used in the treatment of hypertension. The plant has been reported for its antifungal, anticonvulsant, analgesic, anti-inflammatory and androgenic activities and for the treatment of anemia. The leaves are traditionally used in ayurveda system of medicine to bring sound refreshing sleep when it is applied on head about half an hour before bathing. A paste of the root is applied to swellings and

is also used as a rubefacient. Sap is applied to acne eruptions to reduce inflammation. Decoction of leaves used for its mild laxative effects. (Shruthi et al; 2014)

For snakebites, crushed plant is applied topically at place of bite. Juice of leaves is used to get relief from constipation in pregnant women and a decoction has been used to alleviate labour.This is also useful in gonorrhea and balanitis (inflammation of the end of the penis).(Anupama;2015)

Antimicrobial Activity

This study has revealed the presence of secondary metabolites like Steroids and triterpenoids in the leaves and stem of Basella rubra. It has further confirmed that the leaf extract could be used for the treatment of infections caused by the microorganisms E.coli, Pseudomonas aeruginosa, Bacillus subtilis and Aspergillus flavus. (Krishana priya et al; 2015)

Antidiabetic activity

Glucose levels were found to be significantly increased after STZ administration, and there after decreased by administration of Basella rubra. Decrease in serum glucose may be due to the regeneration of beta cells of the pancreas, which were destroyed by STZ. Administration of Basella rubra extract produced a significant ($p<0.01>$ decrease in the blood glucose as compared to diabetic rats. (Nirmalax A et al; 2011)

Antiulcer activity

The aqueous extract of B. rubra possesses significant and dose dependent anti-ulcer and cytoprotective effects against ethanol and pylorus ligated induced ulcer rats and the study was compared with ranitidine (50 mg/kg p.o.) as standard drug. It concludes the results indicate the aqueous extract of Basella rubra in the treatment of gastric ulcers. (S. Deshpande et al; 2003)

7. MATERIALS AND METHODS

Materials

The experimental plants were the leaves of Basella rubra.

Collection and identification of the tested plants

Basella rubra

Basella rubra leaves were procured on December 2016 from plants growing in south india of theni, and dried at room temperature.

The seeds of *Basella rubra* (Basallaceae) were collected from local source, Tamil Nadu (Chennai) in the month of March. The leaves were identified and authenticated by Prof. P.Jayaraman, Retd Professor of Presidency College and Director of Institute of Herbal Science Plant Anatomy Research Centre, West Tambaram, Chennai-600045. A voucher specimen was deposited at C.L.Baid Metha College of Pharmacy for future reference.

Preparation of extracts of Basella rubra

Extraction involves the separation of bioactive portion of the plant tissues from the inactive components by using selective solvents in standard extraction procedure**.**

The powdered leaves (500g) were extracted by using acetone by sequentially extracted using petroleum ether, chloroform, acetone and ethanol in Soxhlet apparatus. Where as a known weight of the coarsely powdered part of each plant was extracted successively with chloroform (60 -80 c°) and ethanol (98%) using soxhlet extractor. The chloroform extract was kept a side and the ethanolic extracts were filtered, evaporated, dryness using Rota vapor apparatus (under

reduce pressure), and the yields were calculated. The residue obtained was kept in dry clean bottles till used (Harborne, 1973).

7.1. PRELIMINARY PHYTOCHEMICAL STUDIES:

The various extracts *Basella rubra* of obtained were subjected to qualitative analysis to test the presence of various phytochemical constituents like alkaloids, carbohydrates, glycosides, flavonoids, steroids, terpenoids, phenols, proteins, tannins etc.

1. Tests for Alkaloids

Mayer's test

A pinch of dried extracts was taken and 2 ml of dilute hydrochloric acid was added, mixed filtered. To the filtrate, one or two drops of Mayer's reagent were added. Formation of pale yellow precipitate indicates the presence of alkaloids.

Dragendorff's test

A pinch of dried extracts was taken treated with 2ml of 2% Acetic acid, mixed thoroughly and filtered. To the filtrate 2 drops of Dragendorff's reagent was added. Formation of orange-brown precipitate indicates the presence of alkaloids.

Hager's test

A pinch of dried extracts was taken and treated with drops of Hager's reagent. Formation of yellow precipitate indicates the presence of alkaloids.

Wagner's test

A pinch of dried extracts was taken and treated with drops of Wagner's reagent. Formation of brown precipitate indicates the presence of alkaloids

2. TESTS FOR SUGAR AND CABOHYDRATES

Molish's test

A small quantity of extracts was dissolved separately in 4ml of distilled water and filtered. Filtrate was treated with 2-3 drops of 1% alcoholic α-napthol solution and 2ml of concentrated

sulphuric acid was added from the sides of the test tube. Brown ring at the junction of two liquids indicates the presence of carbohydrate.

3. TESTS FOR GLYCOSIDES

Anthrone test

A pinch of extracts was taken in a watch glass and 2 drops of alcohol was added to extract. An equal quantity of anthrone was added and mixed thoroughly and dried. Then one drop of concentrated sulphuric acid was added, separated in a thin film with a glass rod in a watch glass.

and heated over the water bath. Formation of dark green color indicates the presence of glycosides.

Test for anthraquinone glycosides

Borntrager's test

A pinch of the extracts was boiled with dilute sulphuric acid, filtered while hot and filtrate was extracted with solvent like benzene. It was shaken well and the organic layer was separated and to this equal volume of dilute ammonia was added. Rose pink colour in ammonia layer indicates the presence of anthraquinone glycoside.

Test for cardiac glycosides

Legal's test

The extracts were hydrolysed for few hours in a water bath. The hydrolysate was added with 2ml of pyridine, sodium nitropruside solution and was made alkaline with sodium hydroxide solution. Orange colour shows the presence of cardiac glycoside.

4. TESTS FOR PROTEINS

Small quantity of extracts was dissolved in a few ml of water and subjected to the following test:

Biuret test

To the extract solution few drops of biuret reagent (1%CuSO4 and 10% NaOH), 1 drop of Copper sulphate solution and 10 drops of sodium hydroxide solution were added. Purple or violet color shows the presence of proteins.

Million's test

Few drops of Million's reagent were added to the extract solution. Reddish brown color shows the presence of proteins.

5. TESTS FOR AMINO ACID

Ninhydrin test

To the extract few drops of Ninhydrin reagent were added. Purple color shows the presence of amino acids.

6. TESTS FOR SAPONINS:

Foam Test:

1 ml of the ethanol extract solutions were taken in a measuring cylinder. To this, 20 ml of distilled water were added and shake well.

Haemolysis Test:

The ethanol extracts of the plant were spread over a glass slide to form a thin film layer on which a drop of human blood was placed and spread over the extract layer. After 30 minutes, the slide was examined under microscope for change in the structure and shape of red blood cells. Control was always maintained to see the change in red blood cells structure for haemolysis.

7. TEST FOR GLYCOSIDES

Small amount of extracts is treated with distilled water and subjected to molish's test .

To the extracts is hydrolyzed with dilute hydrochloric acid and subjected to Lieberman –burchara's, legal's and borntrager's test to detect presence of different glycosides.

8. TEST FOR REDUCING SUGAR

A small portion of extracts was dissolved in water and treated with Fehling's and Benedict's reagent to detect presence of sugars.

9. TEST FOR PHYO STEROLS

The extracts were heated with solution of alcoholic potassium hydroxide until complete saponification. Then it was diluted with distilled water and extracted with ether. The ethereal extract is evaporated and the residue (un saponifiable matter) was subjected to Liebermann's and Lieberman Burchara's test .

10. TESTS FOR PHENOLIC COMPOUNDS

Ferric Chloride Solution test

The extracts were taken in water and warmed; to this 2ml of Ferric Chloride solution was added. Formation of green colour is due to the presence of phenolic compounds

Lead Acetate Solution test

To the extracts (2ml) lead acetate solution was added separately. Formation of precipitate indicates the presence of phenolic compounds.

11. TESTS FOR TANINS

A pinch of the dried extracts was dissolved in ethanol, mixed thoroughly and filtered. The filtrate is tested for the presence of tannins by the following test;

Ferric Chloride

To the filtrate dilute Ferric chloride solution was added.Formation of greenish blue precipitate is due to presence of tannins.

Lead Acetate

To the filtrate lead acetate solution was added(10%). Formation of white colour precipitate is due to the presence of tannins.

Gelatin Solution

To the filtrate 1% solution of gelatin solution containing 10% Sodium Chloride was added. Formation of white colour precipitate is due to the presence of tannins.

TESTS FOR TERPENOIDS

Noller's test or Salkowshi test

A pinch of dried extract in a test tube was taken and a bit of Tinfoil and 0.5 ml of thionyl chloride was added. It was heated gently. Formation of the pink colour is due to the presence of terpenoids.

11. DETERMINATION OF FIXED OILS AND FATS

Spot test

A small quantity of various extracts was pressed separately between two filter papers. Oil stain in the filter paper indicates the presence of the fixed oils.

12. TESTS FOR STEROIDS

Liebermann's Burchard test

The extract were dissolved in 2ml of chloroform and 10 drops of acetic anhydride, 2 drops of concentrated sulphuric acid were added. Formation of green colouris due to the presence of phytosterols.

Salkowski test

A extract was treated with chloroform and a few drops of concentrated sulphuric acid was added along the sides of the test tube. The lower layer of the chloroform showed red colour due to the presence of steroids.

Test for gums and mucilage's

Above 10 ml of aqueous extract was prepared and added to 25 ml of absolute alcohol with constant stirring resulting solution is observed for precipitate.

Test for volatile oil:

The powdered material was subjected to hydro distillation to separate volatile oil if present

7.2. Separation and Isolation of plant Constituents by Chromatographic Methods

Column chromatography

Column chromatography is a separation technique, when a column of stationary phase is used. the technique is called as column chromatography. Based on the nature of stationary phase, whether it is solid or liquid, it is called column adsorption chromatography or column partition chromatography. Column adsorption chromatography is widely used technique.

A. Principle

A solid stationary phase and a liquid mobile phase are used and the principle of separation is adsorption. When a mixture of components dissolved in the stationary phase is introduced in to the column, the individual components move with different rates depending upon the relative affinities. The compound with lesser affinity towards the stationary phase (adsorbent) moves faster and hence it is eluted out of the column first. The one with greater affinity toward the stationary phase (adsorbent) moves slower down the column and hence It is eluted later. Thus the compounds are separated. The type of interaction between the stationary phase and solute is reversible in nature. The rate of movement of a component (R) is given as follows;

This equation can be simplified as follows:

$$R_f \text{ value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Stationary Phase: The stationary phase is one of the two phases forming a chromatographic system. It may be a solid, a gel or a liquid. If a liquid, it may be distributed on a solid. This solid

may or may not contribute to the separation process. The liquid may also be chemically bonded to the solid (Bonded Phase) or immobilized onto it (Immobilized Phase).

Mobile Phase: A fluid that percolates through or along the stationary bed, in a definite direction. It may be a liquid (Liquid Chromatography) or a gas (Gas Chromatography) or a supercritical fluid (Supercritical-Fluid Chromatography). In gas chromatography the Carrier gas may be used for the mobile phase.

B. Column characteristics

The material of the column is mostly good quality neutral glass since solvents, acids or alkalies should not affect it. An ordinary burette can also be used as column for separation. The column dimensions are important for effective separations. The length-diameter ratio ranges from 10:1 to 30:1, For more efficiency the length: diameter ratio can be 100:1. The length of column depends upon

- Affinity of compounds towards the adsorbent used.
- Number of compounds to be separated.
- Type of adsorbent used
- Quantity of the sample

C) Column packing material

The selection of suitable column packing materials is made on the basis of the chromatographic process. Based upon their adsorbent activity, they can be classified as weak, medium, strong adsorbents. They are

Weak	Medium	Strong
Sucrose	$CaCO_3$	Silica gel
Starch	$Ca_3(PO_4)_2$	Activated alumina
Insulin	$MgCO_3$	Activated charcoal
Talc	MgO	Activated magnesia
Na_2CO_3	$Ca(OH)_2$	

D) Solvent selection

The choice of solvents will naturally depend on the first place upon the solubility relations of substance. The solvents commonly used as mobile phase can be arranged according to their increasing eluting power. Elutropic series given below serve as guide for sequential solvent selection, most of the solvents have sufficient low boiling points to permit ready recovery of eluted material.

Points to be selecting while selecting the solvents

The polarity of the solvent, which is passed through the column, affects the relative rates at which compounds move through the column

Polar solvents can more effectively compete with the polar molecules of a mixture for the polar sites on the adsorbent surface and will also better solvate the polar constituents.

Highly polar solvent will move highly polar molecules rapidly through the column.

If a solvent is too polar, movement becomes too rapid, and little or no separation of the components of a mixture will result

If a solvent is not polar enough, no compounds will elute from the column

Solvent	**Percentage of Extract**
Benzene	**0.01%**
Toluene	**0.00%**
Dichloromethane	**0.00%**
Ethylacetate	**0.00%**
Acetone	**0.01%**
Methanol	**0.01%**
Water	**.0002%**

7.3. Physiochemical analysis

ASH VALUE: (Peter S et al; 2001)

This parameter can be used for the determination of inorganic materials such ascarbonates, silicates, oxalates and phosphates. Heating causes the loss of organic material in the form of carbon dioxide leaving behind the inorganic components. Ash value is an important characteristic of a drug and with the help of this parameter we can detect the extent of adulteration as well as establish the quality and purity of the drug. There is considerable difference in the ash values of different drugs but mostly the difference varies within narrow limits in case of the same drug. The acid insoluble ash consists mainly of silica and high acid insoluble ash thereby indicating the contamination with earthy materials. The water-soluble ash is used to estimate the amount of inorganic elements.

Determination of total ash

About 2gm of air-dried crude drug were weighed accurately in a tarred platinum or silica dish and was incinerated at a temperature not exceeding 450°C until free from carbon. It was then cooled and weighed. The percentage of ash was calculated with reference to the air-dried drug.

Determination of Water-soluble ash

The total ash was boiled for 5 min. with 25ml of water. The insoluble matter was collected in a Gooch crucible or an ash less filter paper. It was washed with hot water and ignited for 15min., at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash. The difference in the weight of the ash represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

Determination of Acid insoluble ash

The ash was boiled with 25ml of 2M HCl acid for 15min. the insoluble matter was collected in a Gooch crucible or an ash less filter paper. It was washed with hot water and ignited. It was then cooled in desiccators and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

EXTRACTIVE VALUES:

The amount of an extract that a drug yields in a particular solvent is often an approximate measure of the amount certain constituents that the drug contains. The drug should be extracted with different solvents in order of their increasing polarity to get the correct and dependable values. Generally petroleum ether, alcohol and water extractives are taken into consideration for fixing the standard of a drug. The petroleum ether extract contains fixed oil. Resin and volatile substances, but when the extract is heated at 105℃ until constant weight, the volatile substances are volatized only resin, colorings matter and fixed oil. Alcohol can dissolve almost all the substances but is generally used for determining the extractive index for those drugs which contain glycosides, resins, alkaloids etc. Water is used for the drugs containing water-soluble substances as chief constituents.

- Water soluble extractive
- Alcohol soluble extractive

Determination of water-soluble extractive

5gm of the powder was macerated with 100ml water in a closed flask for 24 hours, shaking frequently for 6 hours and allowing standing for 18 hours. It was filtered and 25ml of the filtrate was evaporated to dryness at 105℃ and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried drug.

Determination of alcohol soluble extractive

5gm of the powder was macerated with 100ml alcohol in a closed flask for 24 hours, shaking frequently for 6 hours and allowing standing for 18 hours. It was filtered rapidly taking precautions against loss of alcohol and 25ml of the filtrate was evaporated to dryness at 105℃ and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug.

Determination of loss on drying

This parameter is used for determination of moisture content. Loss on drying is the loss in

weight in %w/w determined as per the following procedure:

Procedure:

A weighed stopper glass bottle that has been dried for 30 minutes under the same conditions to be employed in the determination was weighed. The sample was placed into thebottle and the contents were accurately weighed. The sample was distributed evenly to depth no exceeding 10 mm. the loaded bottle was placed in a drying chamber (oven) and the stopper was removed. The sample was dried to constant weight at a temperature of 110℃ in hot air oven. The percentage of loss on drying was calculated with reference to the air-dried drug.

Swelling index

1 gm of the bud was soaked in water for 24 hours and then its weight was weighed again. The swelling index is calculated by using the formula,

$$\text{Swelling index} = \frac{\text{Weight after soaking}}{\text{Weight before soaking}}$$

7.4. Instruments and chemicals:

A wide range of instruments and chemicals were used during the course of this

Study as follow:

Instruments:

- Soxhlet apparatus Quick FIT, EX5/83. ENGLAND.
- Water bath BUCHI 461 SWITZERLAND
- Sensitive balance HF-300G A&D COMPANY, Limited
- Haemacytometer Hawksley, ENGLAND.
- Capillary tubes
- condenser
- Rota vapor apparatus

Chemicals

Ethanol Absolute

Petroleum ether

Silymarin

SGOT enzyme Kit (Giri diagnostic kit pvt ltd)

SGPT enzyme kit (Giri diagnostic kit pvt ltd)

Chloroform (Central drug house pvt ltd) New Delhi

7.4.1. Mechanism of Action of silymarin

The mechanisms which provide silymarin hepatoprotective effects are many and varied, and include antioxidation, anti-lipid peroxidation, enhanced detoxification, and protection against glutathione depletion. (Halim AB et al; 1997)

Stimulation of Liver Regeneration:

One of the mechanisms to explain the ability of silymarin to stimulate the regeneration of hepatic tissue is the increase in protein synthesis in damaged livers. In both *in vivo* and *in vitro* experiments, significant increases in the formation of ribosome and DNA synthesis were measured in addition to the increase in protein synthesis. Interestingly, the increased protein synthesis was only measured in damaged livers (partial hepectomy), not in controls.35 The mechanism of increased protein synthesis is currently not known but some authors speculate silymarin imitates a physiologic regulator, so the silybin fits into a specific binding site on the polymerase, thus stimulating ribosome formation. (Schopen RD et al; 1969)

The potential for stimulation of protein synthesis by silymarin was investigated in malignant liver tissue, and no increases in protein synthesis, ribosome formation, or DNA synthesis were found in malignant cell lines. (Sonnenbichler J et al; 1986)

7.4.2. Alcohol hepatotoxicity

Alcohol is one of the main causes of end-stage liver disease worldwide In the United States, alcoholic liver disease is the second most common reason for liver transplantation (Mandayalu Jamal et al 2004). The Dionysus Study, a cohort study of the prevalence of chronic liver disease

in an Italian population showed that 21% of the population studied was at risk for developing liver damage. Of these, only 5.5 % of the individuals at risk showed actual Signs of liver damage.

About 50 years ago it was believed that alcohol in itself was not toxic, rather that the nutritional deficiencies often accompanying it mere the actual causes of liver damage. However, It was shown by Lieber and De Carli that in rats, alcoholic liver damage developed despite suffcient nutrition (DeCarli and Lieber; 1967). The toxicity of alcohol was later on shun to be related to its metabolism by alcohol dehydrogenases (ADHs) and also to the metabolism by CYP2E1. There is also a component of metabolism by catalase (Zilna, Fialova et al. 2001) The main pathway for ethanol (EtOH) oxidation in die liver IS ADH to acetaldehyde, which is associated with the reduction of NAD to NADH NADH increases in xanthine oxidase activity, which elevates production of superoxide Fialova et al 2001). Metabolism of EtOH by alcohol dehydrogenase Influences the redox status of the liver also in other ways. Enhanced acetaldehyde production after EtOH metabolism decreases hepatic glutathione (GSH) content. The decrease in GSH both due to an increased loss, as well as a lower rate of synthesis (Speisky, MacDonald et al 1985). The absolute majority of EtOH oxidation is by ADH to acetaldehyde and by aldehyde dehydrogenase to acetic acid. However, there is a slight of P450 dependent inducible EtOH oxidation due to the CYP2E1 component. Also, CYP1A2, CYP3A4 and CYP2B families may contribute to EtOH oxidation (Johansson, Ekstrom et al. 1988; Lieber 2004).

The first indication that not only alcohol dehydrogenases participate m the metabolism of ethanol came in the early 1970's, when it was discovered that microsomal membrane fractions were capable of catalyzing the oxidation of ethanol. These reactions required NADPH and were inhibited by CO, properties that are distinct from those of alcohol dehydrogenases (Liber. Rubin et al;1970). It was then discovered that this activity was due to CYP2E1 and that the enzyme was inducible by ethanol in rats (Ryan Koop et al. 1986; Johansson Ekstrom et al. 1988)

Free radicals have been implicated in alcoholic liver disease in various ways. Mechanism that are thought to be Involved are impairment of antioxidant defenses, as well as production of reactive oxygen by flie mitochondria and the CYP2E1 enzyme, and by activated phagocytic cells. Oxidative compounds then may lead to activation of immune cells to express pro-fibrotic and pro-Lnflam_matoly cytokines. Macrophages produce TNTa In various conditions that cause

oxidative stress (Ahmed, Aronson et al. 2000), as well as IL-1 and IL-6 (Meng and Lowell 1997). Also, oxidative stress leads to the generation of lipid peroxidation products and protein adducts (Ekstrom and Inge1man-Sundberg 1989; Dupont, Lucas et al 1998; Johansson and Inge1man-Sundberg 1985; Albano, French et al 1999), which eventually stimulate a break in self-tolerance and all immune reaction associated with hepatitis (Albano 2006). CYP2E1 Inhibitors have been shown to reduce the formation of lipid peroxidation products (Inge1man-Sundberg Johansson et al 1993). CYP2E1 has also been should be elevated in non-alcoholic steatohepatltis (Weltman, Farrell et al. 1998). Furthermore it has been shown that the increase m cellular oxidative stress by even moderate alcohol consumption may be one of the mechanisms by which alcoholism progression of chronic hepatitis C, as antibodies towards albumin adducted with lipid peroxidation products were greater in consumers (Riga-monti, Mottaran et al; 2003).

It was suggested that CYP2E1 may play a role in alcoholic liver damage as it has been shovm that during ethanol oxidation CYP22E1 produces O2 -and H202 as a result of uncoupling of oxygen consumption with NADPH oxidation (ingeman-Sundberg and Johansson 1984). In the presence of iron catalysts, even more reactive oxygen species (ROS) can be formed, such as the hydroxyl radical, superoxide and hydrogen peroxide(Ingeman-Sundberg and Johansson 1984; Gonzalez 2005; Cederbaum 2006 and Ingeman-Sundberg 1989); Kou Galeotti et al. 1991). These reactive oxygen species (ROS) may lead to elevated levels of lipid peroxidation products, that in turn adducts with cellular proteins. Adducts may also be formed with nucleic acids. Eventually, this will result In cell damage (Cederbaum 2006) It has been shown that the vanous lipid peroxidation products formed lead to an Immune response in human alcoholics, which worsens as disease progresses (Mot-taraw Stewart et al. 2002). Lipid peroxidation products have also been shown to play a part in fibrosis, by able to activate the stellate cells of die liver to increase their production of collagen which has been particularly studied for the lipid aldehyde malondi aldehyde(MDA) (Maher, Tzagarakis et al. 1994).

Liver injury is often zonated within the liver this is the case for many hepatotoxins that primarily damage the perivenous region and also true in the case of ethanol. Notably, ChQ2E1 is particularly induced in regions by ethanol (Johansson Lindros et al. 1990; Fang, Lindros et al. 1998). vitro studies using HepG2 cells transfected to express CYP2E1, both stably and transiently, have show signs of DNA fragmentation and cell death in transfected cells, which is

not seen in controls. EtOH-induced apoptosis was prevented by an Inhibitor of ethanol oxidation Via CP2E1, and also by an antioxidant that prevents lipid peroxidation (wuand Cederbaum 1999) In this cell model it was also noted that glutathione levels were lowered (Wu and Cederbaum 2004)

Although alcohol is undoubtedly a hepatotoxicity still only approximately 20% of heavy drinkers (males) develop alcoholic cirrhosis (Lelbach 1975). Cirrhosis is most often preceded by hepatitis, generally defined by the following morphologic features; hepatocyte necrosis, neutrophil polymorph Infiltrate fatty changes and usually also Mallory bodies (accumulations of hyaline material in damaged hepatocytes). The series of events preceding cirrhosis are commonly; fatty accumulating hepatitis, fibrosis and finally cirrhosis

Immunological reactions play a large role in alcoholic liver disease. It has long been known that chronic alcoholic liver disease as well as hepatitis is associated with elevated serum cytokine levels, which have prognostic value. TNF alpha is associated with increased mortality in alcoholics (Yin, Wheeler et al. 1999) Serum levels of TNF Alpha receptor 2 and IL-8 have been shown to be significantly elevated in alcoholics (Gonzalez-Reimers, Garcia-Valdecasas-Campelo et al. 2007). Liver disease patients have also been shown to have elevated levels of the pro-inflammatory cytokines IL-6 and IL-8 (Gonzalez-Quintela, Vidal et al. 1999) as well as TNF alpha and IL-2. IL-6 and IL8 are also elevated m alcoholic patients without liver disease. Interestingly, also levels of anti-inflammatory cytokines such as IL-IO are elevated in alcoholics with liver disease cirrhosis (Latvala& Hietala et al. 2005; Szuster-Ciesielska Damluk et al. 2000). Clinically, dietary supplementation with SAM to restore glutathione levels, and phosphatidylcholines to restore membrane finction have been successful and proved some favorable effects on parameters of liver damage (Liebr 2005). S-Adenosy1 methionine (SAM) is a precursor of glutathione. The drug N-acetylcysteine NC), also a glutathione precursor, is used clinically to prevent hepatic failure in the case of paracetamol overdose (Lauterburg, Corcoran et al. 1983; Shah and Gordon 2007). The beneficial effect of SAM has also been shown in vanous animal models. In rats exposed to ethanol and LPS, SABA treated animals showed significantly decreased fibrosis, oxidative stress and steatosis. Supplementation also improves liver function SAM also decreases as TGFß mRNA levels and stellate cell activation (Kapaa 2008) SAM has

also been proved to attenuate EtOH induced glutathione depletion and associated mitochondrial lesions m baboons (Lieber 1994).

7.5. Animals

Wistar albino rats (150-200g) used in studies was procured from C.L.Baid metha college of pharmacy, Chennai-97 The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitium*. All the animals were acclimatized for a week before use. The experimental protocols were approved by Institutional Animal ethics Committee after scrutinization. Animals were received the drug by oral gavage tube. All the animals were care of under ethical consideration as per the CPCSEA guidelines (CPCSEA, 2003) with regular inspections of rats (Sharma A et al; 2012)

7.5.1. Acute toxicity studies:

Healthy Wistar albino female rats of sex weighing 100-170 g maintained under standard laboratory conditions were used for acute oral toxicity test according to Organization for Economic Co-operation and Development guidelines 423. Animals were observed individually at least once during first 30 min after dosing, periodically during first 24 h. Observations were done daily for changes in skin and fur, eyes, mucus membrane (nasal), respiratory rate, circulatory signs (heart rate), autonomic effect (salivation, lacrimation, perspiration, urinary incontinence and defecation) and central nervous system (drowsiness, gait, tremors and convulsion) changes (Sharma A et al; 2012)

Determination of acute toxicity (LD50):

14 days single dose oral acute toxicity and gross behavioral study

Number of animals required: 6 rats (male)
Number of groups: 2 groups (3 animals each group)
Dose levels: 4000 mg/kg body weight of the animals.
Study duration: 14 days

Preparation of dose:

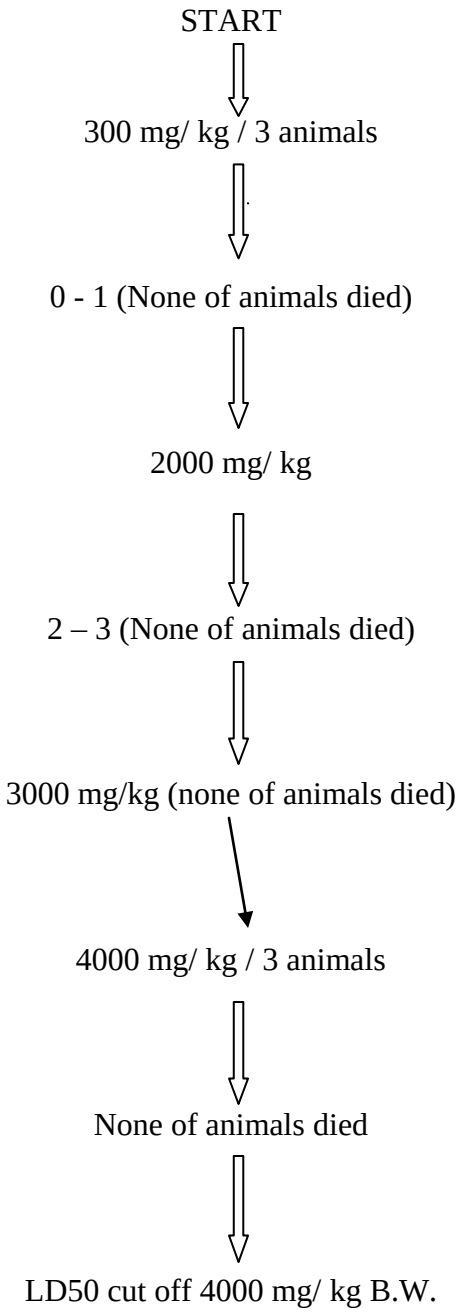
Ethanolic extract of *BASELLA RUBRA* leaves was suspended in 3% CMC, to prepare a dose of 4000 mg/kg body weight of animal, and administered 1ml/100gm body weight of the animal.

Procedure:

The procedure was divided into two phases, Phase I (observation made on day one), and Phase II (observed the animals since next 14 days). Two set of healthy female animal (each set of 3 rats) were used for the experiment. First set animals were divided and fasted for 18 hours deprived from food, water withdrawn before 4 hours of the dosing, body weights were noted before and after dosing with ethanolic extract of *Basella rubra* (4000 mg/kg) orally. Individually animals were observed for 4 hours to see any clinical symptoms, any change in behaviour or mortality. 6 hours post dosing again body weights recorded. Form the next day onwards, each day for 1 hour the behavioral change, clinical symptoms or mortality was observed in the same animals for next 14 days and animal body weights were recorded on 8th and 14th day. The same procedure was repeated with another set of animals to nullify the errors.

FLOW CHART

OECD Guidelines: Test Procedure Starting Dose of 300 mg/kg B.W.



Chemicals

All the chemicals and solvents were of analytical grade. Silymarin was obtained from silybon, Micro Labs, India. Standard kits for SGOT, SGPT and ALP etc. were obtained from Span Diagnostics Ltd., India. Male Albino rats weighing between 150-200 gm used in the experiment were kept in animal house under standard environmental conditions and had free access to feed and water *ad libitum*. The animals were fasted for 16 hours before experiment but allowed free access to water.

The rats were divided into five groups each containing four rats.

Group1	Control	Received water (5ml/kg. p.o) for 21 days once daily, and served as normal control
Group2	Negative control	Received water (5 ml/kg. p.o) for 21 days once daily and 40% ethanol v/v (2.0ml/l00g body wt, p.o.) for 21 days.
Group3	Standard	Received 40% ethanol v/v (2.0ml/l00g body wt, p.o.) for 21 days and standard drug silymarin (25 mg/kg. p.o.) for 21 days once daily
Group4	High dose	Received 40% ethanol v/v (2.0ml/l00g body wt, p.o.) for 21 days and Received ethanolic extract of Basella rubra (400 mg/kg) 21 days once daily
Group4	Low dose	Received 40% ethanol v/v (2.0ml/l00g body wt, p.o.) for 21 days and Received

		ethanolic extract of Basella rubra (200 mg/kg) 21 days once daily

7.5.2. Experimental procedure:

The rats were weighed after the adaptation period and marked with serial numbers and divided randomly into 5 groups, 5 rats each, and then the doses were calculated according to individual body weights.

Blood samples:

Blood was obtained by puncturing retro orbital plexus (Poole, 1989), under anesthesia using Halothane and heparinized capillary tubes. Blood drops were collected, gently, serum was separated by centrifugation (2500 rpm for 15 min), and EDTA was used as an anticoagulant for hematological parameters. Samples were collected before and after dosing with the tested plants extracts at day 0, 5 and at day 10.

7.5.3. Assessment of liver function:

Blood sample were collected into dry clean bottles and allowed to clot for 30 min at room temperature. Serum separated by centrifugation at 2500 rpm for 15 min and stored at -20c° until analyzed. Biochemical parameters, i.e. alanine amino transeferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total bilirubin (TBIL) were analyzed according to the reported methods.

Total Bilirubin:

Total Bilirubin in serum is determined using the method of Jendrassik and

Grof, (1938).

Principle:

Bilirubin in the presence of a sluphanlic acid diazonium salt from a red colored azo compound in alkaline solution. Bilrubin is coupled with diazotized sulfanilic acid in the presence of caffeine to give an azo dye. The optical density was measured by spectrophotometer at wave length 546 nm.

Calculation:

Total Bilirubin is calculated as follow:

Absorbance tube Total × 17.5 = Total Bilirubin (mg/dl).

Alkaline phosphatese:

It is an optimized method according to recommendation of Chemie (1972).

Principle:

In Alkaline medium serum alkaline phosophates splits pnitrophenyl phosphate,

in the presence of Mg+2 ions, into p-nitro phenyl and

Phosphate. At the PH of reaction, p-nitrophenyl was colored yellow, the

optical density measured in a spectrophotometer at wavelength 405nm.

Reaction:

P-nitrophenylphosphate + H2o ALP phosphate + p-nitro phenol

Calculation:

ALP is calculated as follows:

U/1 = 2760 × A 405nm/min

(A = the mean of sample absorbance reading)

Alanine amino transeferase (ALT):

It is an enzymatic method, which measure gultamic pyruive transamine in serum according to Reitman and Frankel (1957), and Schmidt and Schmidt, (1963).

Principle:

Alanine amino transfers is measured by monitoring the concentration of pyruive hydrazone formed with 2-4 dinitrophenylhydrazine .

Reaction:

α - oxoglutarate + L-alanine ALT L-glutrate + pyruivate

The absorbance of samples was read against the reagent blank after 5min at

wavelength 546 nm in spectrophotometer.

Aspartate amino transferase (AST):

It is an enzymatic method that measure gultamic oxaloacetic transaminase in serum according to Reitman and Frankel (1957), and Schmidt and Schmidt,(1963).

Principle:

Aspartate amino transferase is measured by monitoring the concentration of oxaloacetate hydrozone formed with 2-4 dintrophenyl hydrazine.

Reaction:

α- oxoglutarate +L-aspartate AST L-glutarate + oxaloacetate

The absorbance of samples was read against the reagent blank after 5min at wavelength 546 nm in spectrophotometer and cuvette of 1cm light path.

Haematological studies:

Haemoglobin concentration (Hb), packed cell volume (PCV), red blood cells

count (RBC), mean corpuscular volume (MCV), and mean corpuscular

hemoglobin concentration (MCHC), were measured.

Blood samples were collected into dry clean bottles; the anticoagulant was

ethylene diamine tetra acetic acid (EDTA).

Red blood cell count (RBCs):

Total erthrocytes were counted by using Neubauer haemocytometer and Hayem's solution. (Kelly, 1984).

Calculation:

$200 \times 50 \times R$ cells $= 10.000 \times R$ μl.

Haemoglobin concentration (Hb):

The concentration of Hb was measured by the cyanmethaemoglobin method (Kelly, 1984).

The procedure consists of adding 20 µl of blood to 5 ml of a modified Drabkin's solution. After 10 min the solution of cyanmethaemoglobin is compared against a standard in either spectrophotometer (wavelength 540 nm).

7.5.4. Assessment of hepatoprotective activity

After 24 h of ethanol administration, on 22nd day, blood was obtained from animals by puncturing retro orbital plexus. Blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters including SGOT & SGPT (Reitman *et al.*, 1957), ALP (Kind *et al.*, 1954), serum bilirubin (Amour *et al.*, 1965) and serum protein (Lowry *et al.*, 1951) After collection of blood samples, the animals were sacri-ficed under deep ether anesthesia. Morphological parameters like weight of animals, weight of liver have also been used to evaluate the protective effect of the drug. Hepatoprotective chemical causes loss in liver weight/100 gm body weight of rats (Avadhoot *et al.*, 1991; Bhanwra *et al.*, 2000).

6.5.5. Histopathological studies of the liver in paracetamol induced hepatotoxicity

The histopathological evaluation of paracetamol toxicity in all the groups was examined and shown in figure. The description is as follows, Section of rat liver treated with vehicle control group shows liver parenchyma with intact architecture which is the normal appearance. Section of liver in toxicant control group shows partially effaced architecture. Some of the hepatocytes show apoptotic changes, perivenular mononuclear inflammatory infiltration, scattered inflammatory infiltration within the parenchyma which is due to toxicity. Section of liver in silymarin treated group shows liver parenchyma with intact architecture. Some of the central veins show congestion with diffuse congestion of sinusoids.

Section of liver in test drug ethanolqueous treated groups shows intact architecture, few regenerative hepatocytes, sinusoidal congestion and scattered mononuclear inflammatory cells which is similar to silymarin treated group.

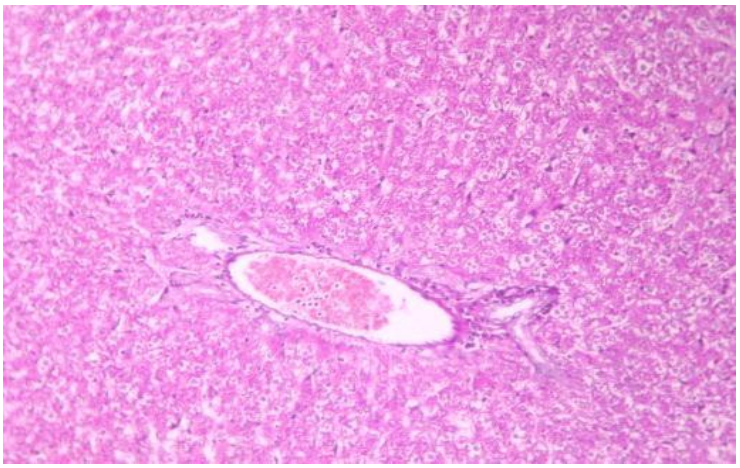


Fig : 1 Normal Contol group, showing normal hepatocytes.

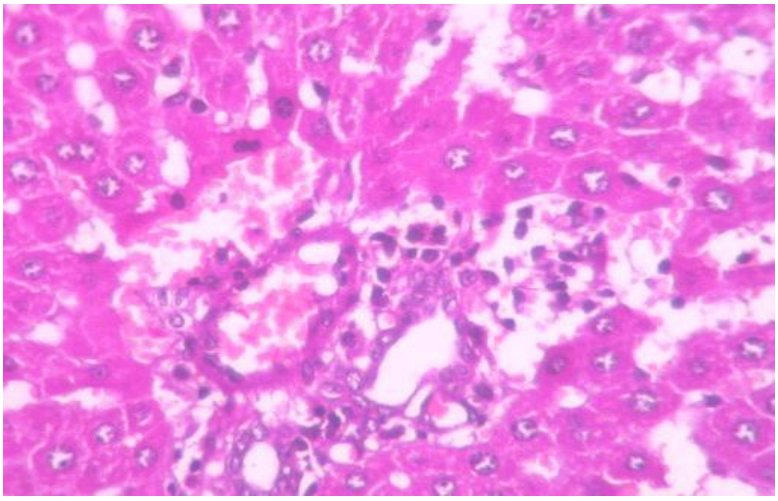


Fig :2 Ethanol treated animal group shows that hepatic cells damage and congestion of the liver.

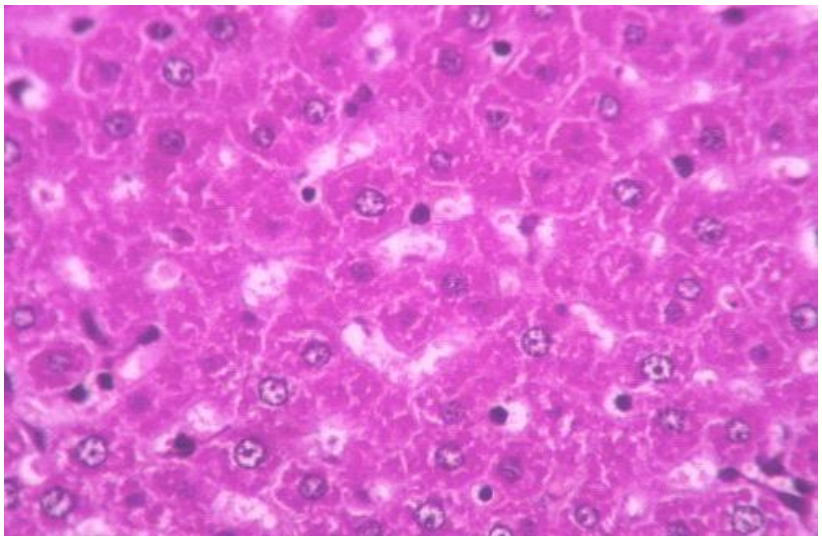


Fig 12 : Hepatocytes in group treated with Standard (Silymarin 200 mg/kg)

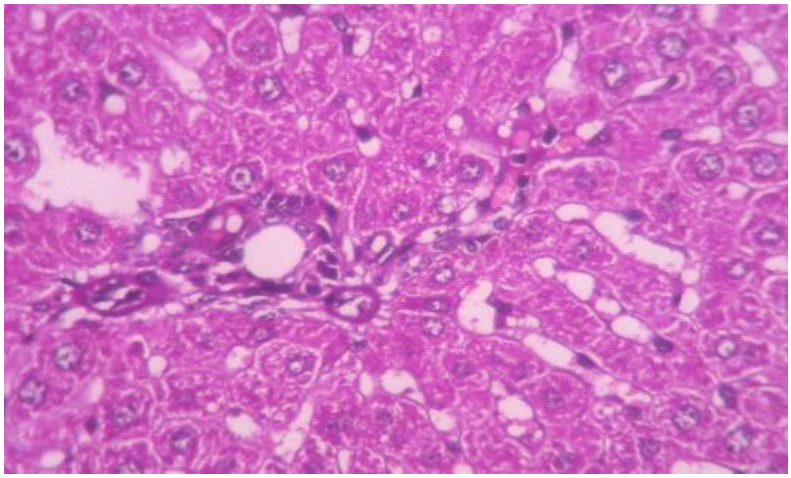


Fig 13 : EEBR of 400 mg/kg shows that few regenerative hepatocytes, sinusoidal congestion and scattered mononuclear inflammatory cells

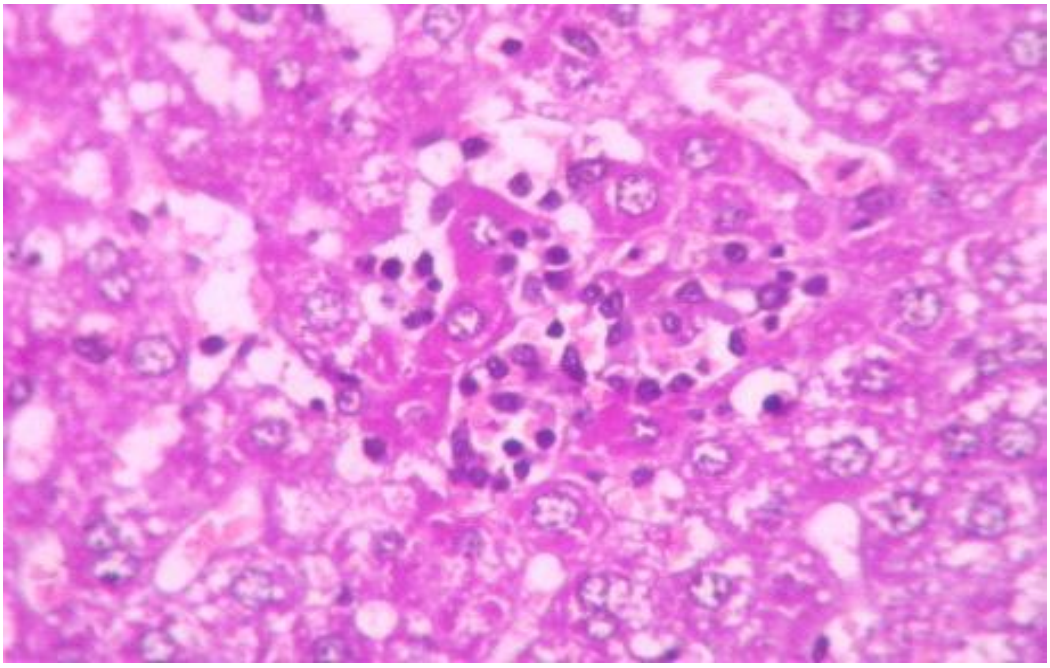


Fig 14: EEBR of 200 mg/kg shows that few regenerative hepatocytes, sinusoidal congestion and scattered mononuclear inflammatory cells

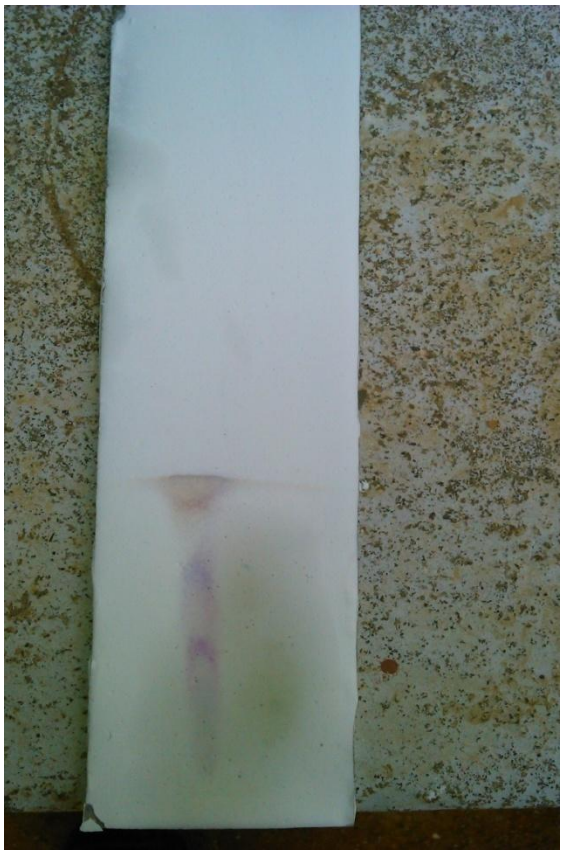
8. RESULTS AND DISCUSSION

Nature of phytoconstituents present in the Basella rubra

1	Test	Result
2	CARBOHYDRATES	+
3	ALKALOIDS	-
4	GUMS AND MUCILAGES	+
5	SAPONINS	+
6	FIXED OILS AND FATS	-
7	TANNINS	-
8	STARCH	+
9	PROTEINS AND AMINO ACIDS	+
10	PHYTOSTEROLS	-
11	PHENOLS	+
12	GLYCOSIDES	-
13	REDUCING SUGAR	+

(+) = indicates the presence of constituents, (-) = indicates the absence of constituents

TLC of ethanol extract of *Basella rubra* leaves.



$$R_f \text{ value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

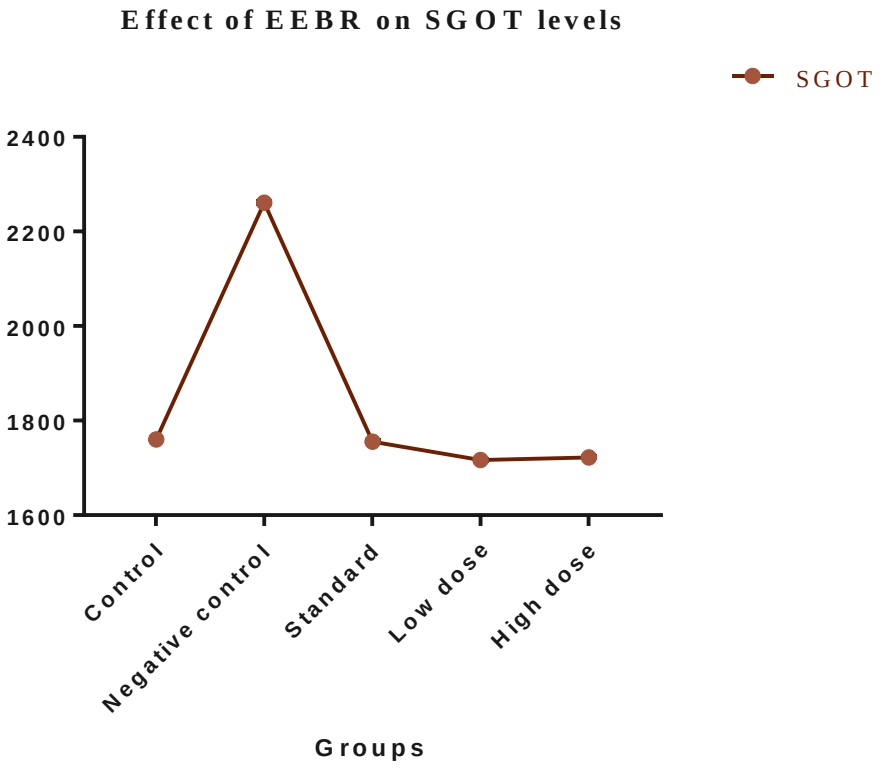
$$= 5.6/10 = 0.56$$

R_f values of ethanolic extracts of Basella rubra leaves

S.No	Extract	Rf value
1	EEBR	0.56

Table 4: Effect of extracts of Ethanolic extract *Basella rubra* leaves on SGOT

GROUP	SGOT level mean ± SEM
Control	1760±1.02
Negative Control	2097.90± 2.468**a
Standard	1759.53±2.33**b
EEBR 200mg/kg	1735.64±1.73 *b
EEBR 400mg/kg	1750.26±1.99 ***b



2.Graphical representation of Effect of EEBR on SGOT

Values are expressed as Mean ± SEM, n=6

Comparison: a -Group I vs. Group II

b- Group II vs. Group III, IV & V; ^{NS} Non significant;

*P<0.05, **P<0.01;***P<0.001

One way ANOVA followed by Dunnet's "t" Test

EFFECT OF EEBR ON SGOT

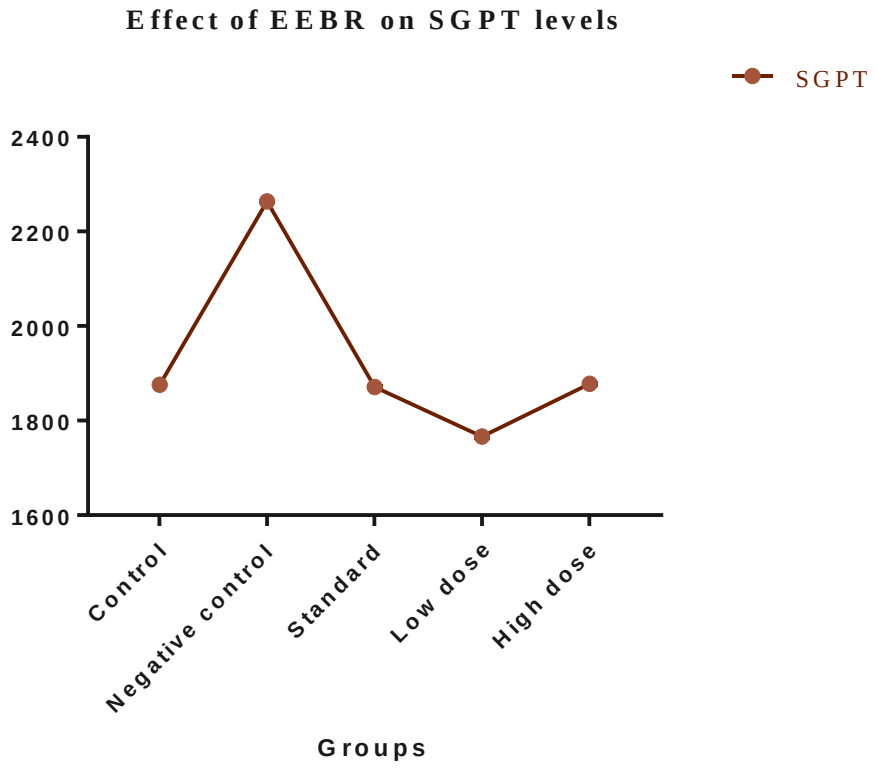
There was significant (p<0.001) increase in serum SGOT in Ethanol induced group when compared to control group. There was significant (p<0.001) decrease in serum SGOT in Silymarin treated group when compared to control group. There was significant (p<0.001) decrease in serum SGOT in EEBR treated group at a dose of 200mg/kg/p.0 when compared to control group. There was significant (p<0.001) decrease in serum SGOT in EEBR treated group at a dose of 400mg/kg/p.o when compared to control group.

There was a significant ($p<0.01$) decrease in serum SGOT level in Silymarin treated rats when compared to ethanol induced. The EEBR at a dose of 200mg/kg/p.o showed a significant ($p<0.010$) decrease in serum SGOT level when compared to ethanol induced group. The EEBR at a dose of 400 mg/kg/p.o showed a significant ($p<0.001$) decrease in serum SGOT level when compared to Ethanol induced group.

The results were shown in the Table no.4 and Graph no.5.

Table 5: Effect of extracts of Ethanolic extract *Basella rubra* leaves on SGPT

GROUP	**SGPT level mean ± SEM**
Control	1875±2.11
Negative Control	2263.36± 1.46**a
Standard	1870.98± 2.65*b
EEBR 200mg/kg	1883.34±2.22**b
EEBR 400mg/kg	2090.66± 2.32**b



3. Graphical representation of Effect of EEBR on SGPT

Values are expressed as Mean ± SEM, n=6

Comparison: a -Group I vs. Group II

b- Group II vs. Group III, IV & V; ^{NS} Non significant;

*P<0.05, **P<0.01;***P<0.001

One way ANOVA followed by Dunnet's "t" Test

EFFECT of EEBR on SGPT

There was significant (p<0.01) increase in serum glutamic pyruvate transaminase level in Ethanol induced rats when compared to control group. There was significant (p<0.05) decrease in SGPT in ethanol treated group when compared to control group.

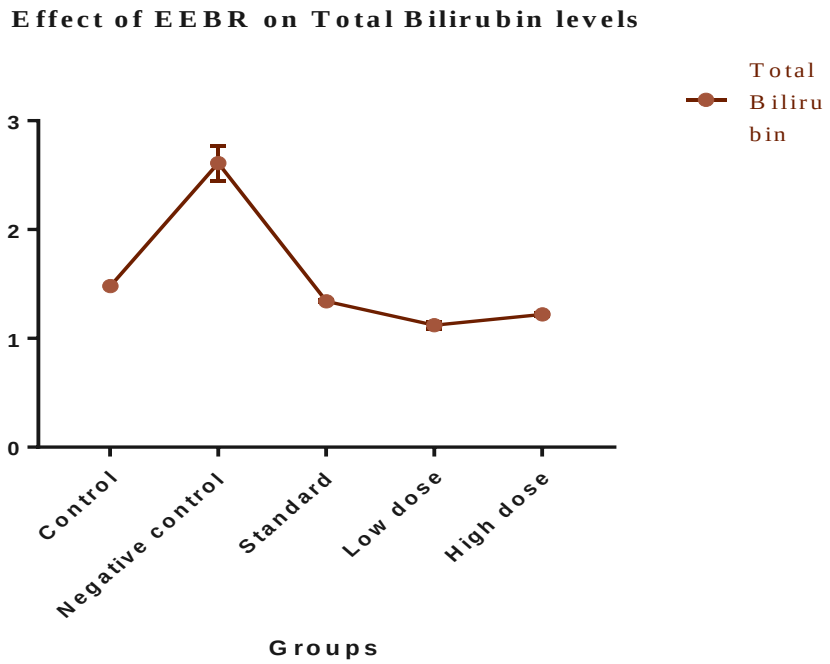
There was significant (p<0.01) decrease in serum SGPT in EEBR treated group at a dose of 200mg/kg/p.0 when compared to control group. There was significant (p<0.01) decrease in serum SGPT in EEBR treated group at a dose of 400mg/kg/p.o when compared to control group.

There was a significant ($p<0.01$) decrease in serum SGPT level in Silymarin treated rats when compared ethanol induced group. The EEBR at a dose of 200mg/kg/p.o showed a significant ($p<0.01$) decrease in serum SGOT level when compared to Ethanol induced group. The EEBR at a dose of 400 mg/kg/p.o showed a significant ($p<0.001$) decrease in serum SGOT level when compared to Ethanol induced group.

The results were shown in the Table no 4. And Graph 3.

Table 5: Effect of extracts of Ethanolic extract *Basella rubra* leaves on Bilirubin

GROUP	Total Bilirubin mean ± SEM
Control	1.48±0.01
Negative Control	2.61 ± 0.16***a
Standard	1.47 ± 0.01*b
EEBR 200mg/kg	1.82 ± 0.03 **b
EEBR 400mg/kg	1.53 ± 0.01 **b



4.Graphical representation of Effect of EEBR on Bilirubin

Values are expressed as Mean ± SEM, n=6

Comparison : a -Group I vs Group II

b- Group II vs Group III, IV & V;^{NS} Non significant;

*P<0.05, **P<0.01;***P<0.001

One way ANOVA followed by Dunnet's "t" Test

EFFECT OF EEBR ON TOTAL BILIRUBIN

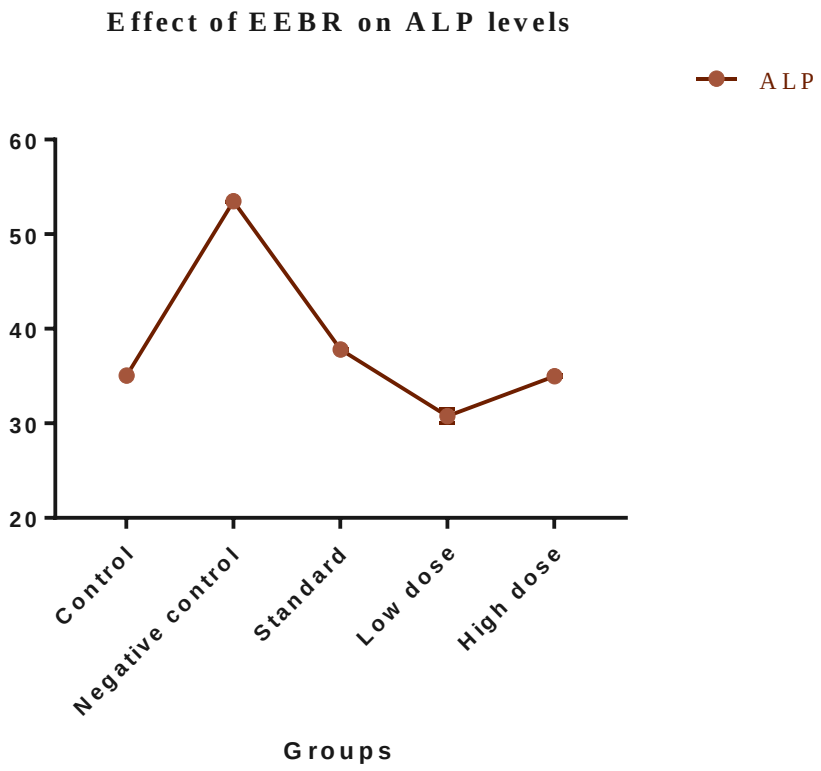
There was significant (p<0.01) increase in Bilirubin level in Ethanol induced group when compared to control group. There was significant (p<0.01) decrease in Bilirubin in Silymarin treated group when compared to control group. There was significant (p<0.05) decrease in Bilirubin in EEBR treated group at a dose of 200mg/kg/po when compared to control group. There was significant (p<0.001) decrease in Bilirubin in EEBR treated group at a dose of 400mg/kg/p.o when compared to control group.

There was a significant (p<0.01) decrease in Bilirubin in Silymarin treated rats when compared ethanol treated. The EEBR at a dose of 200mg/kg/p.o showed a significant (p<0.05) decrease in serum bilirubin when compared to ethanol induced group. The EEBR at a dose of 400 mg/kg/p.o showed a significant (p<0.001) decrease in Bilirubin when compared to Ethanol induced group.

The results were shown in the Table no5. and Graph no.4.

Table 6: Effect of extracts of Ethanolic extract *Basella rubra* leaves on ALP

GROUP	ALP level mean ± SEM
Control	35.06±0.12
Negative Control	53.47±0.01*a
Standard	37.8±0.03**b
EEBR 200mg/kg	35±0.72 ***b
EEBR 400mg/kg	39.98±0.11*b



5. Graphical representation of Effect of EEBR on ALP

Values are expressed as Mean ± SEM, n=6

Comparison: a -Group I vs Group II

b- Group II vs Group III, IV & V;^{NS} Non significant;

*P<0.05, **P<0.01;***P<0.001

One way ANOVA followed by Dunnet's "t" Test

EFFECT OF EEBR ON ALP

There was significant (p<0.01) increase in ALP in ethanol induced group when compared to control group. There was significant (p<0.01) decrease in ALP in Silymarin treated group when compared to control group. There was significant (p<0.05) decrease in ALP in EEBR treated group at a dose of 200mg/kg/p.0 when compared to control group. There was significant (p<0.001) decrease in Alp in EEBR treated group at a dose of 400mg/kg/p.o when compared to control group.

There was a significant (p<0.05) decrease in ALP in Silymarin treated rats when compared ethanol treated. The EEBR at a dose of 200mg/kg/p.o showed a significant (p<0.001) decrease in ALP when compared to Ethanol induced group. The EEBR at a dose of 400 mg/kg/p.o showed a significant (p<0.05) decrease in ALP when compared to Ethanol induced group.

The results were shown in the Table no6. and Graph no.5.

8. DISCUSSION

There are many factors which are responsible for the liver damage or injuries such as chemicals and drugs. In the present study ethanol was used to induce Hepatotoxicity, since it is clinically relevant. Ethanol produces a constellation of dose related deleterious effects in the liver (Leo *et al.*, 1982). The majority of ethanol is metabolized in the liver and individuals who abuse alcohol by routinely drinking 50-60 g (about 4 to 5 drinks) of ethanol per day are at risk for developing alcoholic liver disease (Zakhari *et al.*, 2007). In addition, both acute and `chronic ethanol administration cause enhanced formation of cytokines, especially TNF-alpha by hepatic Kupffer cells, which have a significant role in liver injury (Zhou *et al.*, 2003; Thurman *et al.*, 1998; Tsukamoto *et al.*, 2001). Besides the development of fatty liver (steatosis), another early sign of excessive ethanol consumption is liver enlargement and protein accumulation, both of which are common findings in alcoholics and heavy drinkers (Baraona *et al.*, 1975; Baraona *et al.*, 1977).

Basella rubra are commonly used in the native system of medicine. Various parts of the plant like leaves and roots are medicinally important.

In order to investigate the medicinal use of *Basella rubra* in hepatoprotective, we evaluated crude extract for its Hepatoprotective activity using different *in vitro* assays and *in vivo* rat model of Hepatoprotective activity

Preliminary Phytochemical analysis of ethanolic extract of *Basella rubra* had showed the presence of Phytoconstituents like alkaloids, flavonoids, tannins, saponins and Cardiac glycosides. Flavanoids and alkaloids are widely distributed in the plant which has the property to cure Hepatoprotective activity. Due to this reason the plant has chosen to this study. This shows that the EEBR may contain substances that inhibit liver damage and thus preventing a critical step in Hepatotoxicity

Elevated levels of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) are indications of hepatocellular injury (Yue *et al.*, 2006). In the present study *AEAC* and *AQEAC* at a dose of 500 mg/kg, p.o caused a significant inhibition in the levels of SGOT and SGPT towards the respective normal range and this is an indication of

stabilization of plasma membrane as well as repair of hepatic tissue damage caused by ethanol. On the other hand suppression of elevated ALP activities with concur-rent depletion of raised bilirubin level and an increase in the total plasma protein content suggests the stability of biliary dysfunction in rat liver during hepatic injuries with toxicants (Mukherjee *et al.*, 2002). These results indicate that *AEAC* and *AQEAC* preserved the structural integrity of the hepatocelluar membrane and liver cell architecture damaged by ethanol which was confirmed by histopathological examination.

On examining the liver function tests of ethanol induced animals, the SGOT, SGPT, ALP, Total bilirubin has significantly increased After treatment with the ethanolic extract of Basella rubra (200 mg/kg and 400 mg/kg) the excretion of has SGOT, SGPT, ALP, Total bilirubin significantly decreased Although the low dose was more potent than the high dose when compared with silymarin treated group, which is a standard.

Ethanolic extract *Basella rubra* of has shown promising in vitro efficacy on Hepatoprotective activity, we have observed increase in the absorbance indicating the inhibition of Nucleation and Aggregation of calcium oxalate in in vitro studies.

For the *in vivo* Hepatoprotective activity, of EEBR, Ethanol-induced hepatotoxicity rat model of was used. Since the liver damage inducing treatment, Ethanol, was given orally, therefore, the extract was given p.o. in order to prevent any potential interaction of Ethanol with plant constituents inside gut, interfering with absorption of either of the two. Administration of Ethanol resulted in the increased toxicity, which might be due to the Hepatotoxicity, as evident by increase in SGOT, SG[T, ALP as compared to norma

9. SUMMARY

The present study was aimed to assess the hepatoprotective activity and diuretic activity of methanolic and aqueous extract of *Basella rubra* leaves. LD50 studies were conducted in albino rats with aqueous and methanolic extract of *Basella rubra* leaves according to OECD guideline No.425 and was found safe upto the dose level of 4 gm/kg confirming its non-toxic nature.

HEPATOPROTECTIVE ACTIVITY

The hepatoprotective activity was studied in ethanol induced hepatotoxic animal model. The Physical parameter wet liver weight , Biochemical parameters like serum SGPT, SGOT, and total bilirubin levels, and histopathology of livers were considered as major parameters of study.

Ethanol induced hepatotoxicity was significantly prevented by pretreatment ethanolic extract of leaves. Decrease in wet liver weight, reduction in elevated biochemical parameter levels like serum SGPT, SGOT, and total bilirubin, after treatment with ethanolic extract of *Basella rubra* leaves confirmed the hepatoprotective effect of extract under study. In liver injury models in rats restoration of hepatic cells with minor fatty changes and absence of necrosis after treatment with extract was observed, indicating satisfactory hepatoprotection.

Based on improvement in serum marker enzyme levels, physical parameters, and histopathological studies it was concluded that ethanol extract of *Basella rubra* leaves possesses significant hepatoprotective activity in the doses used.

The hepatoprotective activity was studied in ethanol induced hepatotoxic animal model. The Physical parameter wet liver weight , Biochemical parameters like serum SGPT, SGOT, and total bilirubin levels, and histopathology of livers were considered as major parameters of study.

10. CONCLUSION

HEPATOPROTECTIVE ACTIVITY:

The hepatoprotective effect of ethanolic extract of *basella rubra* leaves was confirmed by the following measures: The isolated livers from the toxicant (ethanol) treated animals exhibited increase in wet liver weight. Indeed, extract treated animals exhibited decrease in the values of above physical parameters as an indication of hepatoprotection. Serum marker enzymes such as SGPT, SGOT and total bilirubin, showed marked increase. The same is observed in liver diseases in clinical practice and hence are having diagnostic importance in the assessment of liver function. In the present study, the methanolic and aqueous extract of *basella rubra* leaves significantly reduced the elevated levels of above mentioned serum marker enzymes. Hence, at this point it is concluded that the methanolic and aqueous extract of *basella rubra* leaves possesss hepatoprotective activity.

In support to this study, histopathological results also show significant activity of the plant. In toxicant treated animals there will be severe disturbances in the cytoarchitecture of the liver. The same is observed in case of humans who are suffering from major liver disorders. But in the methanolic and aqueous extract of *basella rubra* leaves treated group animals exhibited minimal hepatic derangements and intact cytoarchitecture of the liver was maintained. In addition to this there is regeneration of hepatocytes also observed, which indicating Hepatoprotective activity.

Finally based on improvement in serum marker enzyme levels, physical parameters, functional parameters and histopathological studies, it is concluded that the ethanolic extract of *basella rubra* leaves possesses hepatoprotective activity and thus supports the traditional application of the same under the light of modern science.

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ANNEXURE

INSTITUTE OF HERBAL SCIENCE
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AUTHENTICATION CERTIFICATE

Based upon the Organoleptic ✓ /macroscopic ✓ /~~microscopic~~ examination of fresh /market sample, it is certified that the specimen given by G. Meena, M. Pharm, Dept. of pharmacology, C.L. Baid Metha college of pharmacy. is identified as below:

Binomial: Basella alba L. Var. rubra (L.) J.L. Stewart

Family: Chenopodiaceae

Synonym(s): Basella rubra L.

Regional names: Tamil : Shivappupaslakkirai, Kodipasalai.

Reg.No of the certificate: PARC/2017/3461

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